# Early Life Exposure to Antibiotics and Mood and Anxiety Disorders in Children and Adolescents

By

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## Dedication

For my beloved children, Mina and Mohammad, always.

## Abstract

***Objective***: Mood and anxiety disorders are among the most prevalent conditions in children and adolescents with unknown etiology. Exposure to antibiotics in early life has been proposed as a possible risk factor in animal studies. This study was aimed to assess maternal antibiotic use in pregnancy and child antibiotic use in the first three years of life as potential risk factors for subsequent development of these disorders during childhood and adolescence.

***Methods***: A population-based retrospective cohort study was conducted using the Manitoba Population Research Data Repository. The cohort included children born in Manitoba between 1996 and 2012 (221,139 mother-child dyads). Children were followed until the earliest mood and anxiety disorders diagnoses, 19th birthday, migration, death or end of the study period. The antibiotic prescription was identified in the Drug Program Information Network. Physician-diagnosed mood and anxiety disorders were identified using hospital abstracts, medical claims and prescription records. Cox proportional hazard regression was used to compute crude and adjusted hazard ratios (aHRs) with 95% confidence intervals (CI).

***Results***: Children born to mothers who received one antibiotic course or more in pregnancy had significantly higher rates of mood and anxiety disorders compared to children with no exposure (aHR 1.08, 95% CI 1.03 - 1.13) after adjusting for child antibiotic use and other potential confounders. Overall, child antibiotic use during the first three years of life did not increase the risk of developing mood and anxiety disorders (aHR 1.00, 95% CI 0.94 -1.07) after adjusting for maternal antibiotic use and other potential confounders. Children who received tetracyclines, aminoglycosides, quinolones (aHR 1.33, 95% CI 1.24 - 1.43) or sulfonamides and trimethoprim (aHR 1.28, 95% CI 1.22 - 1.34) were at increased risk of developing mood and anxiety disorders.

***Conclusion****:* The modest associations observed for prenatal exposure to antibiotics overall and postnatal exposure to specific kinds of antibiotics may have been confounded by unmeasured factors. While the findings do not support a specific causal relationship between antibiotic use and mood and anxiety disorders due to study limitations, further exploration is merited, and nonetheless, it would be prudent to avoid unnecessary administration of antibiotics.

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**List of Abbreviations**

ADHD = Attention deficit hyperactivity disorder

aHR = Adjusted hazard ratio

ANS= Autonomic nervous system

ASD= Autism spectrum disorders

ATC = Anatomical therapeutic chemical

BDNF = Brain-derived neurotrophic factor

CI = Confidence interval

CIHI = Canadian institute of health information

CNS = Central nervous system

CREB = Cyclic adenosine monophosphate (cAMP) response-element binding protein

CRF= Corticotrophin releasing factor

DALYs = Disability-adjusted life-years

DER-CA = Diabetes education resource for children and adolescents

DPIN = Drug program information network

DSM-V = Diagnostic and statistical manual of mental disorders, fifth edition

ENS = Enteric nervous system

GABA= gamma-amino butyric acid A

GPA=Grade point average

HDAD = Hospital discharge abstract database

HR = Hazard ratio

IBD = Inflammatory bowel disease

ICD = International classification of diseases

ICD-10-CA = International classification of diseases (ICD)-10-Canadian Enhacement

ICD-9-CM = International classification of diseases (ICD)-9-clinical modification (CM)

IQR= Interquartile range

KS= Kolmogorov-Smirnov

LPS = Lipopolysaccharide

MCHP = Manitoba center for health policy

MR= Mental retardation

NGF = Nerve-growth factor

NMDA = N-methyl-D-aspartate

OR= Odds ratio

PH= Proportionality hazard

PHIN= Personal health identification number

PGN = Peptidoglycan

Poly I:C = Polyinosinic: polycytidylic acid

Q = Quintile

Rx = Recipe

SAS = Statistical analysis system

SD = Standard deviation

SEFI-2 = Socioeconomic factor index - version 2

SES = Socioeconomic status

spp. = species

Std. Diff. = Standardized difference

VIF = Variance inflation factor

YLDs = Years lived with disability

WHO = World health organization

# Chapter 1

# Introduction

## 1.1 Introduction and rationale

Mental illness is a "health condition involving changes in thinking, emotion or behavior (or a combination of these) associated with distress and/or problems functioning in social, work or family activities."[1] Two common types of mental illnesses worldwide are mood and anxiety disorders affecting 4.4% and 3.6% of the global population respectively.[2] Mood and anxiety disorders are also listed among the most frequent disorders in children.[3] Although mood disorders are less prevalent in younger children (1% to 2.9% or 1 in 34),[4,5] they can persist into puberty[6] and affect 1% to 12.5% (one in eight) of adolescents.[4,7] Anxiety disorders are identified in 15% to 20% of children and adolescents.[8]

In Canada, 10% to 20% of children and youth are at risk of developing mental health disorders[9] with an estimated of 1.2 million children and adolescents between the age of 9 to 19 suffering from mental illness by 2041.[10] In 2017, approximately 4.5% of Canadian children between the age of 12 and 17 reported that they had received diagnosis of a mood disorder.[11]

In Manitoba, 14% of children were diagnosed with at least one mental health disorder, and the diagnostic prevalence of mood and anxiety disorders among Manitoba's children was 7.3% in 2013.[12] Manitoba also had the third highest rate of self-reported mood disorders (5.7%) among Canadian provinces in 2017.[13]

Mood and anxiety disorders have a significant impact on personal health and social functioning. [2] They can interfere with cognitive abilities, performance at school and the ability to establish healthy relationships with family and peers.[12] Children and adolescents with mood and anxiety disorders are also at increased risk of developing the same diseases later in adulthood as well as physical problems[5,7] such as cardiovascular, gastrointestinal, and respiratory diseases.[14,15] Mood and anxiety disorders are known as the second and seventh leading causes, respectively, of years lived with disability (YLDs).[16,17] These disorders have posed a significant burden on the Canadian healthcare system as 1 in 10 annual users of health care services have these conditions [18] with an estimated cost of $48.6 billion in 2011.[10]

Several biological and environmental risk factors have been associated with the onset of mood and anxiety disorders in children. Some of the proposed elements include genetic,[19-22] social,[19,21,23,24] neurological[19] and perinatal factors[25,26] as well as comorbidities,[27-29] infections,[30,31] parenting[19,23] and family environment[19,23]. Exposure to a "compromised intrauterine environment," such as maternal undernutrition, stress and medications, may also predispose a fetus to develop these disorders later in life (p. 339).[25]

A relevant new risk factor which has come to the forefront of the scientific literature is exposure to antibiotics in early life. Antibiotics are used commonly to treat bacterial infections in both pregnant women and children. Studies show that 37% to 65.8% of pregnant women,[32,33] and 58% of children under the age of 5 receive antibiotics.[34] Two studies conducted in Manitoba, Canada also indicated that approximately 37% of pregnant women[35] and 43.8% of children under the age of 1 year were prescribed antibiotics.[36] Given that in some cases, antibiotic prescriptions are not

necessary,[37] this high exposure and over prescription in prenatal and postnatal periods may lead to short and long-term adverse effects such as immune dysregulation and bacterial resistance, and consequently impact public health.[34,38-40] This also raises the possibility that the risk for developing mood and anxiety disorders in human beings may extend to prenatal and postnatal periods after exposure to antibiotics. Although human research on this topic is limited, animal studies have demonstrated a significant association between early life exposure to antibiotics and depressive and anxiety-like behaviors.[41-44]

Considering the existing gap in the literature for human studies related to early life exposure to antibiotics and mental health, the substantial burden of mood and anxiety disorders, and the high exposure of children to antibiotics in early life (especially in Manitoba), it is important to identify modifiable risk factors for these disorders to inform prevention strategies. Accordingly, this study sought to understand the role of antibiotic exposure in the development of mood and anxiety disorders in Manitoba's children. Chapter 2 provides more detail on the background of mood and anxiety disorders, as well as an outline of the modifiable and non-modifiable risk factors found in the literature.

### 1.2 Empirical objectives

The present study aimed to understand whether there is an association between exposure to antibiotics in early life (prenatal/ postnatal) and subsequent development of mood and anxiety disorders in childhood and adolescence. Accordingly, the following four main objectives and their respective hypotheses were examined throughout this dissertation.

1-To determine if antibiotics prescribed to mothers during pregnancy (prenatal exposure) is associated with an increased risk for the development of mood and anxiety disorders in their offspring during childhood and adolescence.

i- It was hypothesized that prenatal exposure to antibiotics is associated with an increased risk for the development of mood and anxiety disorders during childhood and adolescence.

2- To explore whether there are differences in the risk of mood and anxiety disorders associated with different classes, spectrums, number of courses and timing of antibiotic exposure in the prenatal period.

ii- It was hypothesized that there are differences in the risk of developing mood and anxiety disorders associated with different classes, spectrums, number of courses and timing of antibiotic exposure in the prenatal period.

3- To determine if antibiotics prescribed to children during the first three years of life (postnatal exposure) is associated with an increased risk for the development of mood and anxiety disorders during childhood and adolescence.

iii- It was hypothesized that postnatal exposure to antibiotics is associated with an increased risk for the development of mood and anxiety disorders during childhood and adolescents.

4- To explore whether there are differences in the risk of mood and anxiety disorders associated with different classes, spectrums, number of courses and timing of antibiotic exposure in the postnatal period.

iv- It was hypothesized that there are differences in the risk of developing mood and anxiety disorders associated with different classes, spectrums, number of courses and timing of antibiotic exposure in the postnatal period.

# Chapter 2

## Background and Literature Review

This chapter will provide an overview of mood and anxiety disorders followed by basic information about child population in Canada and corresponding rates of mood and anxiety disorders in children. Next, the chapter explores contextual and individual factors contributing to the development of mood and anxiety disorders. This is followed by background information on antibiotics, microbiome, brain and their relationships. The chapter will be finished with a literature review of animal and human studies on the association between antibiotic exposure and the risk of developing mood and anxiety disorders.

### 2.1 Overview of mood and anxiety disorders

Mood disorders in children are classified as depressive or bipolar.[45] The main characteristics of depressive disorders are a low mood or feeling sad, diminished interest in usual activities and a variety of biological symptoms including sleep disturbance, temper outbursts, decreased concentration, fatigue, recurrent thoughts of death, self-harm and suicide.[4,46] According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V), depressive disorders in children consist of major depressive disorder, persistent depressive disorder and disruptive mood regulation disorder.[4] Bipolar disorders are characterized by manic or depressive episodes.[47] In the manic phase, patients feel increased energy, decreased need for sleep, restlessness and elevated mood while symptoms in the depressive phase are similar to clinical depression such as low mood and reduced strength.[12,47,48] Mood disorders are highly comorbid with anxiety disorders as 62% to 67% of individuals with mood disorders suffer from anxiety

disorders at the same time.[49,50] There is also evidence suggesting the comorbidity of depression with attention deficit hyperactivity disorder (ADHD), conduct disorder and substance use disorders.[4]

Anxiety disorders are characterized by excessive fear, anxiety, fatigue, sleep disturbance and often avoidance of negative and anxiety-producing situations.[12,51] The most prevalent forms of anxiety disorders in children and adolescents consist of separation anxiety disorder and panic disorder.[52]

Research has suggested a temporal relationship between the occurrence of mood and anxiety disorders. Anxiety disorders usually precede mood disorders, suggesting that mood disorders can emerge after anxiety disorders have already begun,[53] but the reverse pattern is also possible.[54] This means that each of these disorders can be a risk factor for one another. There is also the possibility that the onset of both disorders to be at the same time. In this respect, Cummings et al. proposed a model describing three pathways for the development of these disorders.[55] In the first pathway, anxiety disorders develop before mood disorders. The second pathway suggests the simultaneous development of both disorders which could be explained by shared etiology or the overlapping of some risk factors. The third pathway points to the development of anxiety disorders after the onset of mood disorders. These authors identified the first pathway as the most tested and acceptable pathway for the temporal sequence of these disorders in children and adolescents.

Some researchers believe that mood and anxiety disorders are separate entities with conceptually related constructs that share some similar symptoms such as sleep disturbance and fatigue.[51,55] However, the overlapping symptoms of mood and anxiety disorders make a complex diagnosis. The ICD-10 coding system recognizes this issue and considers a separate category for mixed anxiety and depressive disorder (i.e. F41.2).[56,57] The ambiguity and lack of specificity in diagnoses distinguishing between these disorders are problematic for studies using administrative data.[58] Also, shared risk factors (e.g., low socioeconomic status, family stressors) have been identified in the literature for the development of these conditions.[59] Accordingly, some researchers recommend combining these disorders when conducting administrative data studies.[58]

### 2.2 Canadian perspective

On January 10, 2019, approximately 37 million people were living in ten provinces and three territories of Canada[60] where the health care system is publicly funded.[61] The 1984 Canada Health Act stipulates that any Canadian resident is entitled to public health insurance coverage and can receive inpatient or outpatient care from any publicly funded facility at no cost.[61] Provincial health plans also cover outpatient physician visits.[61] Except for children, additional services such as Pharmacare, ambulance and optometric services depending on the province may have no coverage or partial coverage.[61]

On July 1, 2016, there were an estimated 7,865,725 children (22.4 % of the population)[*] in Canada.[62] Of those, 5,839,565 were children aged 14 and younger, and 2,026,160 were

[*]Per author's calculation using data from Canada Census

adolescents aged 15 to 19. In 2016, there were 326,260 children aged 0 to 19 who were living in Manitoba accounting for over one-quarter (25.5%)* of Manitoba's population, close to 1 in 4 Manitobans.[63] Accordingly, the proportion of children in Manitoba is higher than Canada overall. Manitoba's Healthy Child Committee of Cabinet also reported that the number of Manitoba's children overall has increased in the past decade (2006 -2016) by 6% and it was expected that between 2013 and 2020 the population of children under the age of five would increase by 11%.[64,65]

The rise in the number of children, especially in Manitoba should be taken into account as children are more likely to develop mental health disorders compared to adults.[66,67] According to a Canadian Institute of Health Information (CIHI) report in 2015, 10% to 20% of Canadian children were vulnerable to develop mental health disorders.[9] It was also estimated that by 2041, 1.2 million children and adolescents between the age of 9 to 19 will suffer from a mental health disorder.[10] In 2012, the highest rate of mood disorders (8.2%) was reported among the age group 15 to 24 years old.[66] In 2017, approximately 4.5% of children between the age of 12 and 17 reported receiving a diagnosis with a mood disorder.[11] In the same year (2017), and within the same age group (12 to 17 years old), Manitoba had the third highest rate of self-report mood disorders (5.7%) in Canada[13] after Alberta (6.9%)[68] and British Columbia (6.8%)[69]. In 2016 - 2017, about 8.6% of adolescents (1 in 12) who were residents of British Columbia, Saskatchewan and Manitoba reported that they had been prescribed at least one medication for treatment of mood and anxiety disorders.[70]

*Per author's calculation using data from Canada Census

The prevalence of mood and anxiety disorders was higher when using administrative health databases in the following studies[12,71] compared to data collected through self-reporting in previous reports.[13,70] This discrepancy may be partially related to different or overlapping age groups, time of study and/or information biases (e.g., recall or recording). For instance, over a four-year study period (2009 - 2013), 14% of Manitoba's children between the age of 6 and 19 had been diagnosed with at least one mental health disorder, and the diagnostic prevalence of mood and anxiety disorders among them was 7.3%.[12] According to a Manitoba Center for Health Policy (MCHP) report in 2005/2006, 11% of Manitoba's children 0 to 19 years of age received at least one medication for depression in a given year.[71] The rate of anxiolytic drug prescriptions in Manitoba's children aged 0 to 19 had also been increasing over a study period from 5.0/1000 in 2000/01 to 6.1/1000 in 2005/06.[71]

This increasing rate of mood and anxiety disorders is of concern because of its impact on individual health and social functioning. Irrespective of the age groups, the global burden of mental illness was estimated at 13% of disability-adjusted life years (DALYs) and 32.4% of years lived with disability (YLDs).[16] According to a 2017 World Health Organization (WHO) report, mood and anxiety disorders accounted for 567 to 844 YLD per 100,000 populations living in the American region including Canada.[2]

Mental health disorders are also a major economic burden for the Canadian healthcare system with an estimated cost of $48.6 billion in 2011 and an expected $2.5 trillion in 2041 due to health care, social services, lost productivity, income support and reduced health-related quality of life.[10] According to a Canadian report, three-quarters of annual health care services were for

mood and anxiety disorders.[18] This report indicated that children aged 5 to 14 years showed the highest increase in the rate of health care utilization over a 14-year period (1996/97 to 2009/10).[18,72*]

Another report showed that urban children aged 10 to 17 with a diagnosis of mood and anxiety disorders accounted for the highest rate of hospital service use in 2015 with 51% increase in emergency department visits and 48% increase in inpatient hospitalization over an 8-year period (from 2006/07 to 2013/14).[9]

Additionally, depression is highly associated with suicidal death[4] which in turn has economic impacts.[10,73] In 2010, the total cost of self-harm in Canada and Manitoba were $2,956 and $114 million respectively.[74†] Aside from the human costs, the high rates of suicidal death among children and adolescents has posed a significant burden on the Canadian economic and health care system due to lost productivity, police services, autopsy, disability and hospitalizations[75,76] with a total cost[‡] of $385 million in 2004.[77] Suicide is also a significant public health problem in Canadian children, given that it was the leading cause of death[78] among children aged 10 to 14 (19%),[79§] and the second cause of death[80] among children aged 15 to 19 (27%)[81**] in 2016.

[*] Using text description of Figure.1 in Canadian Chronic Disease Surveillance System data files, for the age group 5 to 9 years old, it was 5.9 % to 7.9%. For the age group 10 to 14 years old, it was 6.0% to 8.6%.
[†] For general population
[‡] Per author's calculation using data from Table # 16 for age groups 0 to 19 reported in the reference (p.28)
[§] Per author's calculation using data from the related table in the reference (47 cases in 242 deaths)
[**] Per author's calculation using data from the related table in the reference (185 cases in 687 deaths)

### 2.3 Contributing factors to the development of mood and anxiety disorders

The development of mood and anxiety disorders depends on the interplay of the child's individual characteristics and his/her circumstances.[19] To explore the pathways to the development of mood and anxiety disorders, we need to focus on both individual risk factors and broader contextual factors. Contextual factors exist in a range of circumstances consisting of biological, family and school contexts.[19] In the following section, these factors will be reviewed.

#### 2.3.1 Contextual factors

The most studied pathways in the development of mood and anxiety disorders are related to the biological context. There is substantial evidence that some neural structures are implicated in mood and anxiety disorders. The amygdala, a region in the sub-cortex, has a role in mediating fear, anxiety, and emotional memory.[82,83] It is also a part of a neural circuit consisting of the mesolimbic dopamine system, prefrontal cortex, and hippocampus.[84] While the mesolimbic dopamine system is known as a mediator pathway for reward and pleasure,[85] the prefrontal cortex has a role in controlling behavior.[86] Prefrontal cortex dysfunction and left frontal underactivity have been associated with depression.[87-89] The septo-hippocampal area of the brain has also been involved in the development of anxiety.[90,91] There is also evidence that the hippocampus and amygdala in both depressive and anxious children have larger volumes than in non-affected children.[92] This could be one reason why anxiety and depression are so highly comorbid in children.

Another neurological vulnerability to mood and anxiety disorders is the dysfunction of the hypothalamic-pituitary-adrenal axis. This axis regulates cortisol in human stress responses via

affecting the pituitary and adrenal glands.[93,94] The improper regulation of cortisol has been linked to depression.[95]

Within the biological context, genetics is considered a contributing factor for depression and anxiety.[19] Genetics plays its role in the form of either gene involvement (genetic influence) or heritability (genetic predisposition).[19] To date, no specific genes have been identified in the development of mood and anxiety disorders, but there is some evidence that these disorders have heritable and familial bases with a variance approaching 65%.[15,22,96-98] For instance, children whose first-degree relative has an anxiety disorder, are four to six times more likely to develop the same disorder.[22] Children with depressed parents are also three times more likely to demonstrate a depressive episode compared to their counterparts.[21,98] Accordingly, familial basis or heritability which implicate a genetic predisposition is known as a strong predictor of depression and anxiety in children.[19]

The effect of genetics on the development of mood and anxiety disorders may be mediated by another biological construct known as temperament.[99-101] Temperament is a relatively stable trait with a genetic basis which manifests in early childhood.[19] Perceiving the world as hostile, threatening, or stress-inducing, is a common characteristic of anxious and depressed people who feel angry, low self-adequacy, and being under greater stress.[19,102] Anxious people also have an inhibited temperament which is regulated by a behavioral inhibition system. This system is sensitive to punishment and threat.[19,91] In anxious and depressive children, this system becomes overactive.[103,104] As a result of this hyperactivity, the child becomes more vigilant and cautious and develops a threat perspective when processing information.[19] In the presence of genetic

susceptibility, the child tends to show more avoidant behavior when confronting stressful events.[15] Living with over-controlling parents makes this situation even worse as the child has less opportunity to learn adaptive behaviors.[19] Their interactions with family members and peers are also limited, and any conflict or criticism can easily hurt them and cause a sense of insecurity and difficulty in managing emotions and reactions.[15,105] When these children are exposed to repeated stress and fail to adapt to adverse situations, they feel hopeless and are vulnerable to developing mood disorders.[15,106]

In addition to parenting styles (i.e., controlling type), there are other factors related to the family context which play a role in the pathway to the development and maintenance of mood and anxiety disorders. The highly influential factors include adverse family life events, chronic stress, family abuse, low family support,[107] childhood adversities and experience of sibling loss, family dissolution or parental loss through death or separation.[21,108,109]

The school context is another pathway for the development of mood and anxiety disorders in childhood and adolescence**.** Children spend many hours at the school where they have the opportunity to develop interpersonal skills via social interaction and relationships.[19] However, these social interactions can be demanding and induce anxiety in children by negatively affecting their coping skills, learning and academic performance.[19] Accordingly, the school can be viewed as a source of stress.[19] There is also evidence that social and academic failure can result in self-incompetency and depression.[19,110] On the other hand, depressive children tend to exhibit lower cognitive ability, which in turn causes academic achievement problems.[19,111] Additionally, there is an interaction between relationships with peers and academic performance which both act as

predictors of depression in children.[112] A study showed that low-grade point average (GPA) could predict depressive symptoms only in children who have a relatively low number of friendships.[112] It seems that "competency in one domain can moderate the risk in the other domain" (p. 68).[19] While social and academic factors can function independently, their combination and interaction can contribute to the development of child mood and anxiety disorders.

### 2.3.2 Individual factors

Individual risk factors for mood and anxiety disorders can be classified as modifiable and non-modifiable. Investigators are more interested in identifying modifiable risk factors as they can be addressed and changed via interventions. Recognizing the risk factors that cannot be changed is also crucial for diagnostic purposes and the assessment of disease progression. For instance, children can be categorized into different categories of a variable (e.g., sex), and the possibility of developing mood and anxiety disorders for each group (male, female) can then be assessed. Then, clinicians can use this information to modify their diagnostic criteria as well as the assessment of the disease progression.

#### 2.3.2.1 Non-modifiable risk factors

Biological sex is one of the non-modifiable risk factors for the development of mood and anxiety disorders.[15,21] Females are often known to be at higher risk for developing depression.[113] This phenomenon is attributed to biological susceptibility and sociocultural issues.[114] In adolescents, a sex difference was reported in the levels of depressive symptoms, with girls being more affected than boys.[115] Bradley believed that mood and anxiety disorders affect both men and women

equally, but among adolescents, females are at higher risk for developing depressive disorders compared to boys.[15] A recent study also showed a higher risk of developing mood (Odds Ratio: OR=2.99) and anxiety (OR=2.29) disorders among female adolescents aged 10 to 21 years.[21]

There are also some perinatal factors associated with the development of mood and anxiety disorders. For instance, older maternal age (35 years and over) has been linked to depression and anxiety in adult female offsprings.[116] However, Hyland et al. did not find any association between parental age and childhood mood and anxiety disorders.[21]

Research has also shown an association between vitamin D deficiency with the development of depression in the general population[117] but with no evidence in children. Animal models have suggested that vitamin D can prevent hippocampal cell death induced by glucocorticoids and therefore may play a role in glucocorticoid signaling which is dysregulated in depressive disorders.[118,119] Maternal deficiency in vitamin D and other nutrients that affect the immune system may increase the risk of infection in mothers[120] and subsequently may increase the risk of mood and anxiety disorders in offspring.

Another potential risk factor is the season of birth. Literature supporting the role of seasonality in the development of mood and anxiety disorders in children is lacking. However, one study reported that spring and summer seasons were strong determinants of depression in adulthood that could be attributed to nutritional deficiency or other unknown in-utero factors.[121]

The association between multiple births and the development of mood and anxiety disorders later in childhood and adolescents has not been well examined. However, research suggests that compared to mothers of singletons, mothers with multiple births are at higher risk of developing postpartum depressive disorder which is a potential risk factor of childhood depression.[21,122]

There is also substantial evidence supporting the association between preterm birth and psychiatric disorders.[26,123,124] A meta-analysis reported an increased risk of developing anxiety in adolescents who were born as preterm**.**[123] In a national Canadian cohort study on 3732 infants, Colman et al. also found that those who were born small or large for gestational age manifested increased levels of depressive (50%) and anxious (31%) symptoms in adolescence**.**[124] Some studies specifically listed low birth weight among perinatal problems as early childhood risk factors for developing depression.[125-127]

Evidence supporting the role of residence location as a risk factor is still controversial. For instance, it has been reported that urban residents experience depression 39% and anxiety 21% more frequently than rural residents.[128] In contrast, one study found no difference in the rate of mood and anxiety disorders between urban and rural settings.[129] None of these studies focused on childhood mood and anxiety disorders. However, there is evidence supporting the role of residence location in the development of childhood anxiety disorders but not in mood disorders. [21] In a large cohort study, Hyland et al. showed that urbanicity was significantly associated with a 17 % increase in the risk of anxiety disorders in children aged 10 to 21 years old. Their study did not identify urban dwelling as a significant predictor of mood disorders.[21]

Another risk factor lacking sufficient evidence in the literature is breastfeeding. A study reported an association between breastfeeding status and the child's social, attention, and aggression problems in early adolescence.[130] There is evidence showing breastfeeding as an independent predictor of overall mental illnesses in children and adolescents.[131,132] The shorter duration of breastfeeding was conversely associated with increased mental health problems.[131] However, Kwok et al. failed to find any significant associations between breastfeeding and early adolescent behavioral problems, self-esteem and depressive symptoms.[133]

Socioeconomic status (SES) and its relation to economic, social and physical environments are known to be a source of stress.[134] Consequently, it has the potential to contribute to the development of mood and anxiety disorders in children.[19,135] The American Psychological Association (APA) defines socioeconomic status as "the social standing or class of an individual or group"[136] with three indicators: education, income and occupation.[137] Costello et al. examined age, sex, place of residence, race and income as potential correlates of childhood psychiatric disorders.[138] Income as an indicator of SES was the strongest predictor in their study. They found a higher rate of depression and other disorders in low-income children, and the most impoverished children were three times more likely to develop a mental health disorder. In a study of 796 preschool children, Hopkins et al. measured SES using a four-factor index (education attainment, occupation prestige, marital and employment status) and found that SES was indirectly correlated with symptoms of anxiety and mood disorders through mediating variables such as conflict, stress, child temperament, parental depression and parenting style.[24]

#### 2.3.2.2 Modifiable risk factors

There is evidence linking mood and anxiety disorders with both bacterial (e.g. tuberculosis and pneumococcus)[139-141] and viral (e.g. influenza, herpes simplex virus, human immunodeficiency virus,[142] hepatitis C virus,[142,143] varicella-zoster virus,[142,144] human T-cell lymphotropic virus[145]) infections in general population. Viral and bacterial infections have also been proposed as potential risk factors for developing mental health disorders in children. In a cross-sectional study of 39 children between the age of 5 to 12, Wilson et al. described a combination of symptoms including anorexia, anxiety, unexplained crying with clinging and fear of death that significantly differed among children with serological evidence of Coxsackievirus B compared to those with no evidence of infection.[146] Blomstrom et al. also conducted a large cohort study consisting of 1,172,879 children and found a 23% increase in the risk of developing psychosis after hospitalization due to bacterial infections in Central Nervous System(CNS). The authors did not report the type of bacterial agents in their study.[31] Similarly, a population-based retrospective cohort study of 96,020 children showed a 2 % increase in the risk of depression after being infected with enteroviruses including Coxsackievirus B.[30]

These associations suggest that pathogenic agent by itself or its effect on the immune system may have been involved in the onset or course of the mental disease. Pathogenic agent components can influence signaling to the brain and affect its function.[147] They can produce pro-inflammatory cytokines, which can cross the blood-brain barrier and affect the metabolism of neurotransmitter, function of the neuroendocrine system as well as synaptic plasticity in the CNS.[148] Infection with enteric pathogens can also alter microbiome composition and intestinal permeability.[149] It is believed that elevated levels of pro-inflammatory cytokines as a marker of

immune dysregulation result from interactions with the gut.[150] Accordingly, infections may affect the mental status of the host via interacting with gut microbiome and inducing a pro-inflammatory response at systemic and CNS levels.[148] On the other hand, depression and anxiety have been linked to the neuroinflammatory process as an increased level of pro-inflammatory cytokines has been documented in individuals affected with these disorders.[151] The potential mechanism that contributes to this association was explained by Gareau in his microbiome-gut-brain, cognition theory[149] and will be discussed in detail in Chapter 3.

In addition to infection as the target of antibiotic therapy, many individuals diagnosed with mental health disorders have one or more comorbid chronic diseases. For instance, depression is one of the most common psychopathologies of asthmatic patients that reduces their quality of life and increases their consumption of corticosteroids and admission to hospital.[152-154] Anxiety and depression have also been associated with diabetes,[155,156] inflammatory bowel disease (IBD),[157,158] atopic dermatitis,[159] seizure disorders,[160,161] hypothyroidism,[162] and cancer in general population.[163] There is also evidence suggesting a linkage between depression in children and chronic diseases such as asthma,[28] diabetes,[27] IBD,[29] cancer,[164] atopic dermatitis,[165] and seizure disorders[166]. While these associations remain poorly understood, it is well documented that not only are depressive and anxious people more vulnerable to develop chronic physical illnesses but also those suffering from chronic diseases are at increased risk of developing mood and anxiety disorders.[18]

It has also been suggested that some medications can contribute to the development of mood and anxiety disorders in the general population via their neurotoxic side effects. Drugs that are

recognized for these effects and have been implicated in inducing depression include: isotretinoin (acne medication), alpha interferons (for treatment of hepatitis C and some types of cancer), corticosteroids (anti-inflammatory agent), varenicline (smoking cessation agent), progesterone inserts (contraceptive agent), finasteride (for treatment of benign prostatic hyperplasia and androgenic alopecia),[167] rimonabant (anti-obesity drug).[168] The manifestation of the depressive effect of these medications depends on the vulnerability of the individual.[169] Several pathophysiological mechanisms have been proposed for the depressive side effects of these medications. Most of these mechanisms were explored in animal studies and are as follows: a) reducing the number and size of neurons, number of cholinergic receptors and the activity of G-protein-coupled catecholaminergic,[170] b) affecting the release of dopamine[171] and serotonin produced in hippocampus[172] c) disturbing cholinergic-adrenergic balance,[171] and finally d) affecting mesolimbic dopaminergic system.[171]

Antibiotics were also reported in 2011 as having been added to the list of medications with depressogenic effects [173] which will be discussed in the next section.

### 2.4 Antibiotics, microbiome and the brain

#### 2.4.1 Overview of antibiotics

Antibiotics are prescribed to treat bacterial infections[174] and have a critical role in the maintenance of human health. They are the most widely prescribed medications in pediatrics.[34] One study indicated that the prevalence of antibiotics prescription in childhood differs across age with the highest rate in preschool children.[175] In another study, 58% of children under the age of 5 were prescribed antibiotics.[34] A Canadian study reported that 43.8% of Manitoba's one-year-

old children received at least one antibiotic course.[36] Yet, in 50% of children, antibiotic prescriptions are not necessary.[37] This overprescribing may cause short and long-term adverse effects such as immune dysregulation and bacterial resistance and consequently, impact public health.[34,38-40]

Antibiotics are also listed in the top twenty most frequently used medications in pregnant women.[176] Depending on the setting, about 37% to 65.8 % of pregnant women receive systemic antibiotics during the prenatal period,[32,33] and more than 40% are prescribed antibiotics immediately before delivery.[33,177,178] For instance, in a rural area in Ghana, as a developing country with a high prevalence of cesarean deliveries (84%), the rate of antibiotic prescription was 65.8% in whole pregnancy, 75% in the third trimester and 42.4% within 24 hours to the childbirth.[33] While in a developed country such as Denmark with 22% prevalence of cesarean sections, the antibiotic exposure rates were 37% in the whole pregnancy, 18% in the third trimester and 33% near delivery.[32] The variation in the reported prevalence of antibiotic exposure across studies may be related to different rates of prenatal infection, prophylaxis administration for cesarean section as well as social and lifestyle factors.[32,33,177]

Canadian studies revealed that 36.8% of mothers received at least one course of antibiotics during pregnancy,[35] and more than 20% were administered antibiotic prophylaxis for group B Streptococcus during parturition.[179] Notably, maternal antibiotic use in pregnancy can result in fetal exposure as eleven types of antibiotic can cross the placenta.[176]

There are different types of antibiotics. The WHO Collaborating Centre for Drug Statistics Methodology classifies antibiotics using Anatomical Therapeutic Chemical classification system (ATC) codes and updates it once a year (**Appendix 1**).[180]Antibiotics are also classified as either narrow-spectrum antibiotics, which work against specific (types of) bacteria or broad-spectrum antibiotics which act against a wide range of bacteria (**Appendix 2**).[181] Based on the mechanism of action, antibiotics are categorized into three main groups. As described in **Figure 1,** the first group acts on the bacterial cell wall and prevents its synthesis such as β-lactams, bacitracin, and vancomycin.[182] The second group affects the bacterial protein synthesis by targeting bacterial ribosome and inhibiting the biosynthesis of either 30S (e.g. aminoglycosides, tetracyclines) or 50S (e.g. macrolides, oxazolidinones) subunits of these proteins. The third group of antibiotics targets bacterial DNA and inhibits its replication (e.g. quinolone). As well, sulfonamides and trimethoprim are included in this group which acts upon folic acid metabolism and inhibits its synthesis.

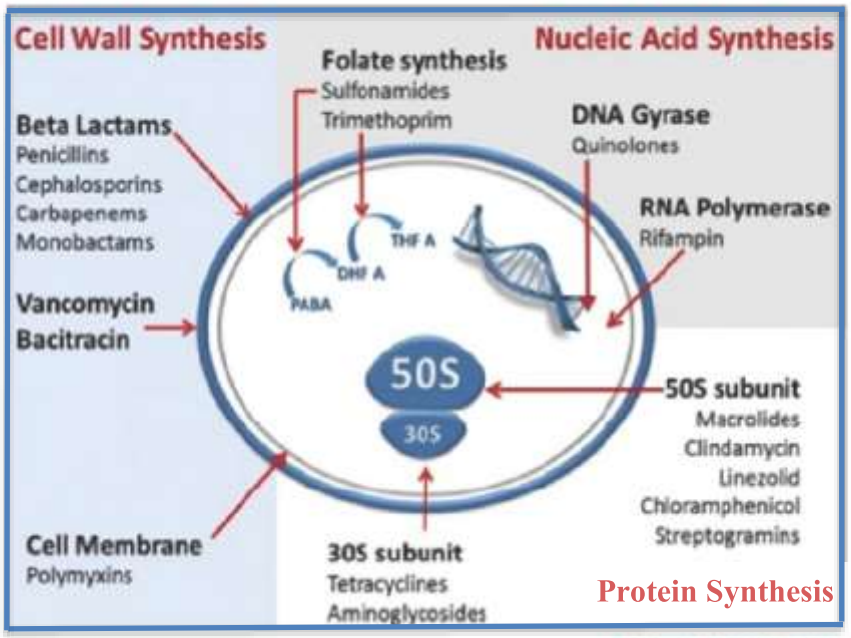
Besides targeting bacterial pathogens, antibiotics can directly alter brain function and cause a wide variety of psychiatric side-effects.[173,183-187] For instance encephalopathy, seizure and behavioral changes have been reported after exposure to penicillins,[173] metronidazole, ofloxacin, cotrimoxazole and clarithromycin.[183] Treatment with quinolone, sulfonamides and trimethoprim,[173] as well as ciprofloxacin[183] have also been associated with psychosis.

Notably, the manifestation of these neurotoxic effects depends on age, interaction with other medications and the ability to cross the blood-brain barrier.[186] The most common recognized mechanism responsible for these adverse effects is acting on gamma-aminobutyric acid A

(GABA).[173,188] For instance, β-lactam penicillin can inhibit the activity of GABA receptors, induce endotoxins and cytokine release as well as increase glutamate.[173,188] Macrolides and quinolones also demonstrate their neurotoxicity effect by deactivating GABA receptors and activating N-methyl-D-aspartate (NMDA) receptors.[173]

The impact of antibiotics on brain function may also result from their high affinity to bind to mitochondria and disrupt its function in brain cells (e.g. tetracyclines, aminoglycosides).[183] This antibiotic-induced mitochondrial dysfunction has been linked to the development of depression.[183] In addition to these direct effects, antibiotics are also known to potentially affect the brain function mainly through shaping the gut microbiome which will be described in the following section.[185]

**Figure 1- Mechanism of action of antibiotics***

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### 2.4.2 Overview of the microbiome

The microbiome is a collection of different microbial cells, genes, and metabolites which inhabit in and on our body.[189,190] The microbiome is normally harbored on the skin, mucous membranes, upper respiratory tract, mammary glands, vagina, uterus, ovarian follicles, placenta, seminal fluid, saliva, external genitalia, external ear canal, outer eye (lids, conjunctiva) and gastrointestinal tract.[191] The large population of bacteria in the gut is called gut microbiome.[147,192] Healthy gut microbiome usually contains a highly diverse population of four main phyla including *Firmicutes* (e.g., *Clostridium, Enterococcus, Lactobacillus, Ruminococcus*); *Bacteroidete* (e.g., *Bacteroides*, *Prevotella*), *Actinobacteria* and *Proteobacteria*.[193,194]

The gut microbiome coexists in a commensal relationship with the body[195,196] and has a role in the function of the gut epithelial barrier, homeostatic mechanism, intestinal angiogenesis, and the adaptive immune functions.[195,197,198]

The gut colonization occurs in the fetal period and has a maternal signature as some studies have indicated the contamination of placenta and the first stool of a newborn with microbes.[199] There is also substantial evidence that the complexity of the microbiome composition which started in fetal life increases during childbirth.[200-202] The birth canal, the living environment and being handled by other individuals are also known as main sources of gut colonization during the first days of life.[185,203,204] It is believed that the colonization of infants born through vaginal delivery results from maternal fecal and vaginal bacteria, whereas in cesarean born infants, initial exposure to the hospital environment and healthcare workers are the primary sources of

colonization.[205,206] Accordingly, the type of delivery is known as an influential factor in the establishment of microbiota and its developmental variations.[179,205,207]

Irrespective of the method of childbirth, the development of gut microbiome continues during the first three years of life and becomes more diverse until it resembles an adult microbiome.[194,207] Accordingly, the prenatal period and the first three years of life (collectively called "early life") when gut microbiome colonization and maturation occurs, are recognized as windows of vulnerability to external perturbations.[208-210]

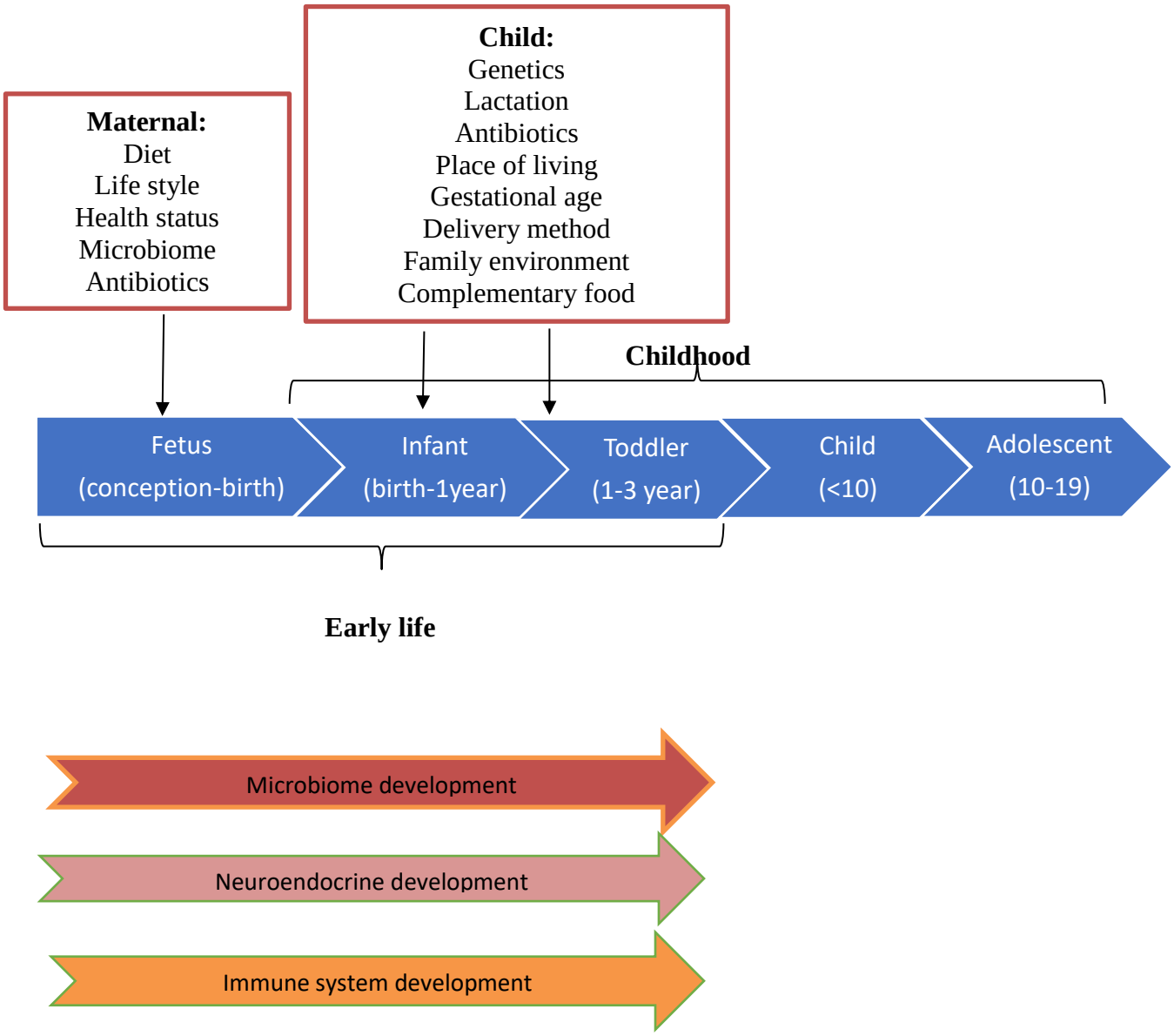
Early life is also a critical period of neurodevelopment when synaptogenesis reaches maximum levels.[185,204] The establishment of the neuroendocrine system,[211] immune systems,[212-216] and metabolic regulation, which is conditional on microbiome maturation, also parallel this period.[217,218]

As depicted in **Figure 2**, many factors influence the development of the gut microbiome during early life including maternal microbiota, lifestyle, diet and health status as well as delivery method, genetics, gestational age, family environment, lactation, complementary food, place of living and antibiotics.[219-223] Therefore early life disruption of the microbiome, called dysbiosis, can be induced by any of these factors.[185] For instance, cesarean delivery has been associated with negative changes in the developing microbiota (i.e. decreasing the number of beneficial bacteria).[222] Blaser et al. also stated that gestational antibiotics might modify the maternal microbiota at the time of birth which in turn may impact infant gut colonization.[221] They also

noted that post-pregnancy antibiotic exposure via maternal milk, or oral secretions when mothers pre-masticate food might involve other mechanisms for microbiome perturbation.

There is also evidence suggesting that dysbiosis has the potential to interfere with the neurodevelopment process via the immune system and the gut-brain axis, and thus may be associated with subsequent mental health outcomes.[185] Notably, dysbiosis is not a phenomenon limited to early life and can be induced by many factors throughout life.[220] These factors include nutritional status,[224,225] lifestyle,[220] age,[220] physical activity,[225] inflammation,[220] stressors,[226,227] alcohol consumption, [228,229]smoking habits,[230] disruption of diurnal rhythm,[231] sleeping patterns, maternal separation, migration and antibiotics[220,232].[147]

**Figure 2- Factors influencing the development of gut microbiome in early life as a critical period.**



#### 2.4.2.1 Microbiome and the brain

The effect of gut microbiota on the animal brain has been shown through neuroendocrine, metabolic and immune pathways.[147,233] Several information carriers such as microbiota-derived signaling molecules, neurons, mediators of the immune system and intestinal hormones play a role.[147] Microbiome metabolites are carried by blood circulation and can either directly reach the brain or interact with the other information carriers.[234-236] This link between gut and brain, known as the gut-brain axis, was not recognized until the 20$^{th}$ century when it was critically studied by prominent researchers in different science fields including physiology, psychiatry, and psychology.[147,237-241]

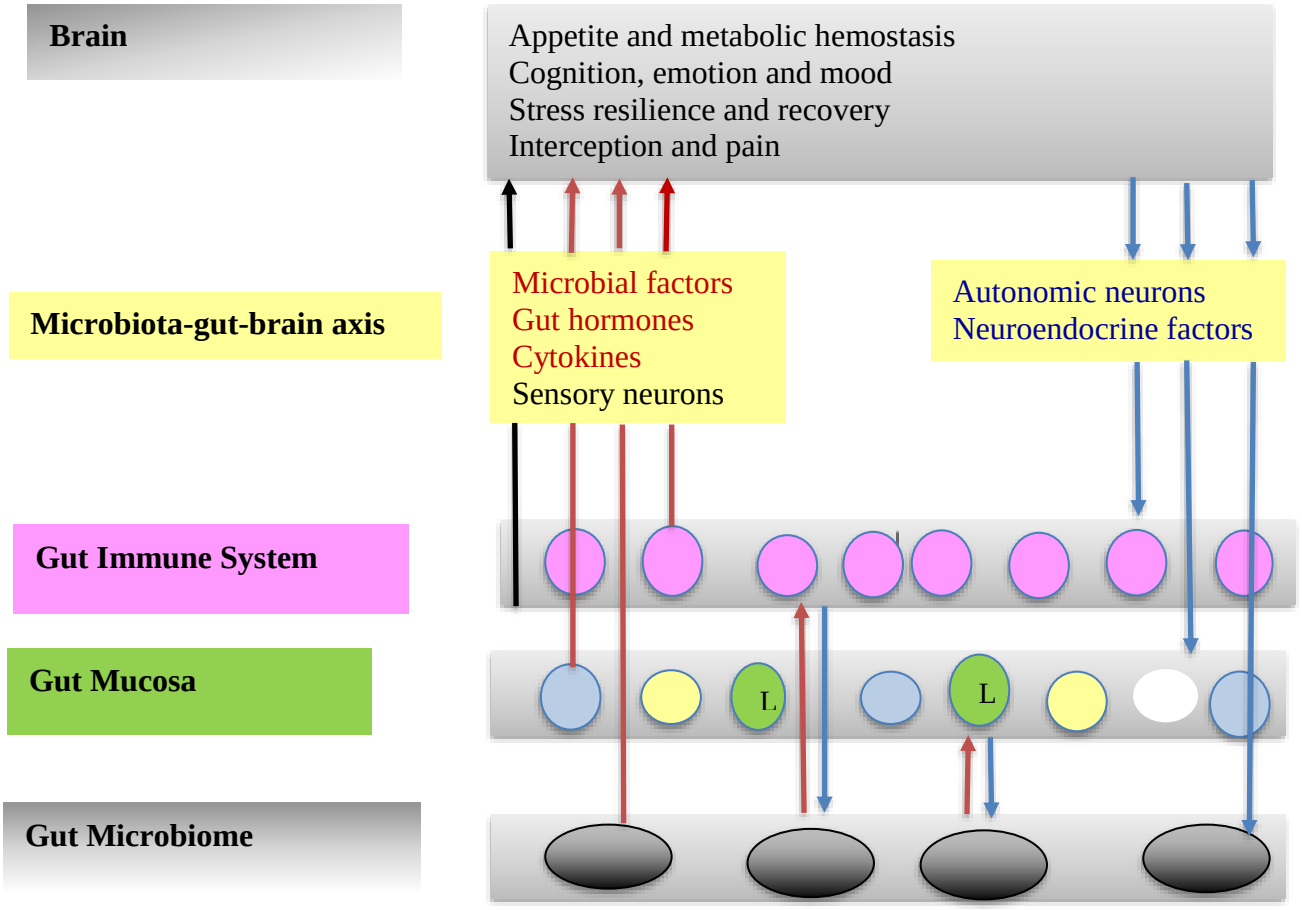
#### 2.4.2.2 Gut-brain-axis

The gut-brain axis is a bidirectional link between the gut and the brain in health and disease.[147] This link is based on four major information carriers including neural messages, immune mediators (cytokines), gut hormones, and microbial metabolites.[234-236] As depicted in **Figure 3,** these four communication pathways can affect cerebral function and behavior by signaling from the intestine to the brain.[147] Autonomic neurons and neuroendocrine mediators are the only pathways that are involved in signaling from the brain to the gut.

Any of the four pathways can involve neuropeptides, known as transmitter molecules in the enteric nervous system (ENS) and CNS that share transduction mechanisms with gut hormones.[147] There is evidence supporting that the microbiome produces these neuroactive components.[147] As presented in **Table 1**, neuropeptides isolated from human gut bacteria include gamma-aminobutyric acid (GABA), serotonin, short and long chain fatty acids, catecholamines

and acetylcholine.[147] Each neuropeptide can enable epithelial cells to produce metabolites which can affect enteric neural signaling or can directly influence the activity of primary afferent neurons to the CNS.[147] Microbiome metabolites and their function will be discussed in the following section.

**Figure 3- The bidirectional microbiota-gut-brain axis***




(Copied and reproduced with permission from Holzer and Farzi,2014; Chapter 9; p.198)[147]
L denotes endocrine L cells in the intestinal mucosa.

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#### 2.4.2.3 Microbiome metabolites

As presented in Table 1, *Lactobacillus* species (spp.) and *Bifidobacterium* spp. produce GABA,[242] which is involved in physiological and psychological functions of the brain.[147] GABA dysfunctions and signaling have been connected to anxiety and depression.[243]

Another metabolite is serotonin (5 hydroxytryptamines, 5-HT) which is produced by *Streptococcus* spp., *Escherichia* spp. and *Enterococcus* spp.[147] Serotonins as a tryptophan metabolite can contribute to the regulation of brain function and mood.[147] *Escherichia* spp. and Bacillus spp. produce norepinephrine.[147]

Dopamine is another metabolite produced by Bacillus spp. and is involved in regulating emotion, motor, cognitive, memory and endocrine functions.[244] Dysfunction of catecholamines, another metabolite of the microbiome, has been associated with major depressive disorders.[245]

*Lactobacillus* spp. can produce acetylcholine and histamine.[246]Acetylcholine is significantly involved in cognition, memory and learning processing.[147]

*Lactococcus, Lactobacillus, Streptococcus* and *Enterococcus* produce histamine, which plays a vital role in the maintenance of wakefulness[247] and cognitive function.[248]

Other microbiome metabolites with neuroactive function are short-chain fatty acids which are involved in cell signaling[249] as well as neurotransmitter synthesis and release.[250] Being able to cross the blood-brain barrier,[147] these type of fatty acids are primary energy sources in brain cells

needed for the early development of this organ [251-253] and can increase synthesis of the dopamine and related catecholamines.[254]

A long-chain fatty acid is also a microbiome metabolite that is involved in the brain's structural and signaling functions as well as neurogenesis and neurotransmission within the nervous tissue. [147,255,256] Several fatty acids, including arachidonic acid and docosahexaenoic acid, are known for their critical role in neurological development.[255,256]

The effect of the microbiome metabolites on the gut-brain axis was further confirmed by animal studies that will be discussed later in this chapter.

**Table 1- Representative list of neurochemicals isolated from bacteria within the human gut***

| **Genus** | **Neurochemicals** |
|---|---|
| *Lactobacillus, Bifidobacterium* | GABA |
| *Streptococcus, Escherichia, Enterococcus, Lactococcus, Lactobacillus* | Serotonin |
| *Escherichia, Bacillus* | Norepinephrine |
| *Escherichia, Bacillus, Lactococcus, Lactobacillus, Streptococcus* | Dopamine |
| *Lactobacillus, Bacillus* | Acetylcholine |
| *Lactococcus, Lactobacillus, Streptococcus, Enterococcus* | Histamine |

(Copied and reproduced with permission from Wall R. et al., 2014; Chapter 10; p.224)[147]

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### 2.4.3 Antibiotics and microbiome dysbiosis

As already mentioned, antibiotics can modify brain function by affecting the gut microbiome.[185] The impact of antibiotic exposure on the microbiome composition of offspring, called antibiotic-induced dysbiosis, lasts from pregnancy[257,258] and intrapartum[179] to the neonatal period.[185,259,260]

There is also substantial evidence in the literature supporting the dysbiosis side effect of some antibiotics that are mainly prescribed for pediatric infections. The first group of antibiotics includes β-lactams. β-lactam antibiotics are a group of broad-spectrum antibiotics, with a beta-lactam ring in their molecular structures and consist of penicillin, cephalosporins and their derivatives.[261] As discussed earlier, this class of antibiotics inhibits bacterial cell wall synthesis.[182,262] In addition to affecting the bacterial pathogens as the primary target of treatment, there is evidence that they can decrease gut bacterial diversity (e.g. ampicillin);[263] as well as the abundance of some bacteria such as anaerobes and enterobacteria (e.g. cefotaxime,[264] amoxicillin,[265] meropenem,[266] ticarcillin).[39,267] In a study, ampicillin, sulbactam and cephalosporins were used to treat Clostridium difficile - associated diarrhea, and the combination of these antibiotics changed the total composition of gut microbiome by reducing the abundance of *Firmicutes* and increasing *Bacteroidetes*.[268]

The second group of antibiotics includes macrolides, lincosamides, streptogramins**.** As discussed earlier, with a similar mechanism of action, all these antimicrobials inhibit protein biosynthesis (50S subunit) in the bacterial ribosome at early stages.[182] Also, they can modify the microbiome.[39] For instance, clarithromycin and metronidazole decrease the abundance of *Actinobacteria*.[269] Another example is exposure to erythromycin that alters the community of

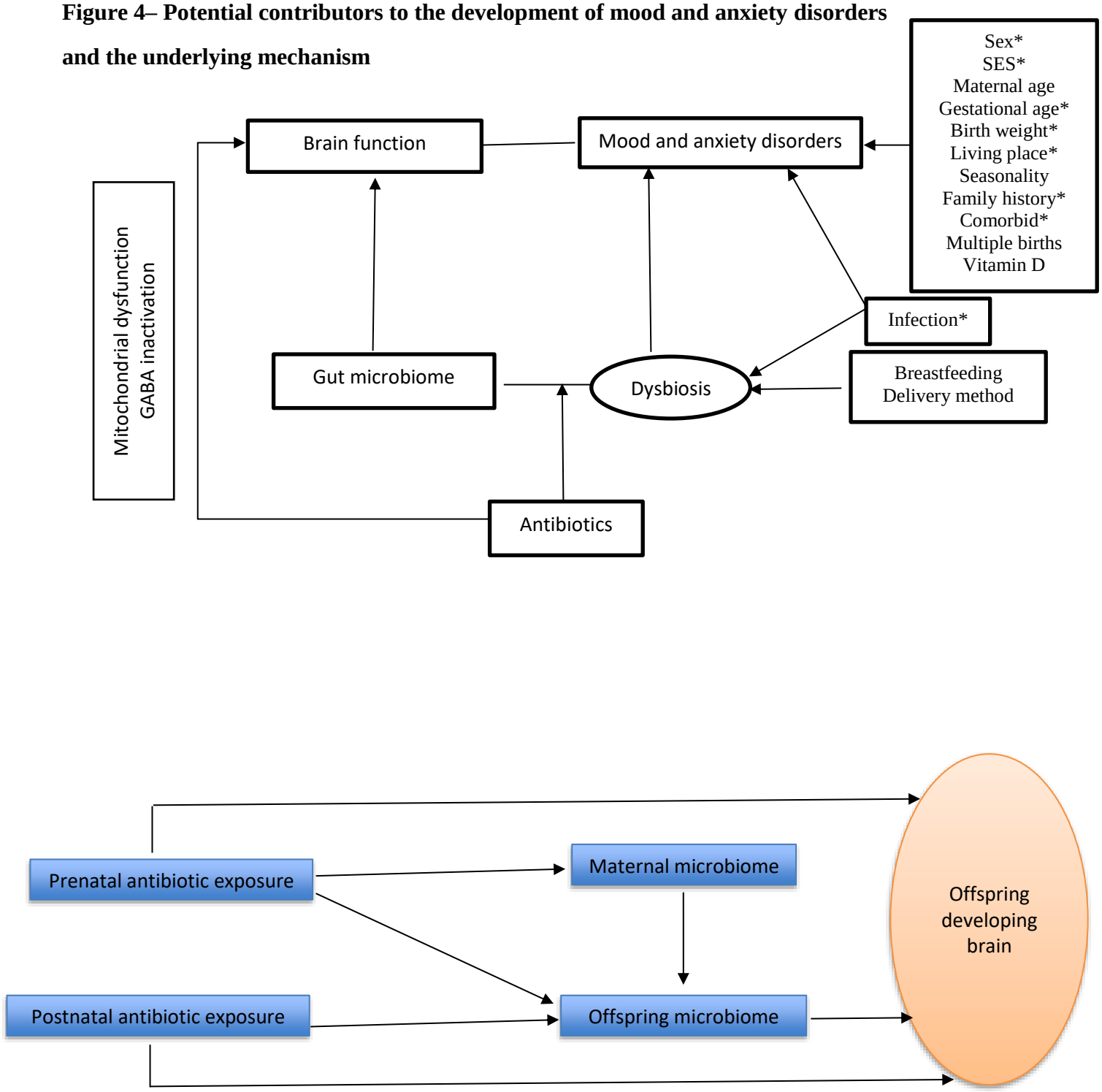
enterobacteria and anaerobes.[270] In a similar vein, lincosamides such as clindamycin initially reduces the abundance of *Enterococci, Streptococci,* and anaerobic bacteria, but this reduction is subsequently recovered.[271] Clindamycin also decreases the diversity of *Bacteroides* spp.[272] and reduces the abundance of bacteria which produce short-chain fatty acids.[39,273]

The last group of antibiotics includes tetracyclines such as tigecycline which inhibits protein biosynthesis (the 30S subunit) in the bacterial ribosome.[182,274,275] Tigecycline is known to affect microbiome by decreasing the abundance of *Enterococci, E. coli, Lactobacilli, Bifidobacteria,* and *Bacteroidetes* as well as increasing other enterobacteria, yeasts and proteobacteria.[276,277]

Taken together, the above information would appear to suggest that exposure to antibiotics in early life has the potential to alter neurological function and microbiome composition of offspring and thus may contribute to the development of mood and anxiety disorders. As presented in **Figure 4**, factors other than antibiotic may contribute to the development of these disorders. As mentioned earlier, the review of the literature suggested that seasonality, vitamin D and medications were associated with mood and anxiety disorders in the general population. More so, biological sex, SES, gestational age, birth weight, family history, living place, infection and comorbid chronic diseases were specifically associated with these conditions in children. While the roles of maternal age and multiple births are still unclear, breastfeeding and delivery method were linked to microbial dysbiosis which in turn can be associated with mood and anxiety disorders. Infections can directly and/or indirectly (via dysbiosis effect) impact the development of mood and anxiety disorders. The evidence also showed that gut microbiome becomes more stable after the age of 3 years compared to infancy (birth to 1 year) and the

toddler period (1 to 3 years). Given that medications may influence microbiome in later life, it is possible that maternal antibiotic use during pregnancy can alter maternal microbiome and subsequently impact the microbiome of offspring. However, most findings are based on research in animals and have not been translated into human studies.

**Figure 4– Potential contributors to the development of mood and anxiety disorders and the underlying mechanism**




*Risk factors identified in literature that were specifically associated with mood and anxiety disorders in children

## 2.5 Literature review on antibiotic exposure and the associated risk of developing mood and anxiety disorders

### 2.5.1 Review of animal studies

Traditionally, insights into antibiotic-induced dysbiosis have been based on comparisons between germ-free mice and colonized mice. In a study, oral administration of a combination of bacitracin, neomycin and pimaricin for ten days increased gut inflammation and caused changes in host physiology such as increased visceral sensitivity and activity of gut inflammatory cells.[44] These changes were reversed by treatment with dexamethasone, and gut function was improved after the restoration of *Lactobacilli*. This study showed that antibiotics had the potential to induce gut inflammation in the absence of normal gut microbiome. This finding also indicated that antibiotic-induced dysbiosis might be the reason for the observed physiological changes in mice.

Using a similar antimicrobial cocktail, Bercik and colleagues lavaged mice with a combination of bacitracin, neomycin, and pimaricin over seven days.[41] The researchers reported anxiolytic behavior in mice which returned to normal within two weeks after cessation of antibiotic treatment. The findings showed a reduction in the level of the brain-derived neurotrophic factor (BDNF) in the amygdala. In contrast, the level of BDNF increased in the hippocampus. All these changes were normalized two weeks after treatment cessation. Notably, administration of the same antibiotics into peritoneum did not affect behavior. This finding suggested that dysbiosis was responsible for changes in BDNF and behavior modification.

To investigate the underlying mechanisms, Bercik et al. also found that the removal of the thoracic vagus nerve by surgery or inactivating sympathetic nerves with chemicals did not affect the antibiotic-induced anxiolytic behavior.[41] Elevations in cytokines in antibiotic mice led these investigators to conclude that behavior changes were related to the direct or indirect effects of the microbiome on the brain. Subsequent experiments by Bercik et al. also supported the idea that the microbiome has a role in behavior alteration.[41] They used microbial components of two different strains of mice for transferring: one daring and courageous; and the other group were shy and timid. The mice who were daring, after being colonized with the other group's microbiome, became less daring while those colonized with the microbiome of daring mice showed more courageous behavior.

In a recent study, the linkage between antibiotic exposure and the development of neuropsychiatric disorders was explored by Leclercq et al.[43] The authors focused on early life exposure to low-dose penicillin in the offspring of mice and showed that penicillin could cause long-lasting changes in gut microbiota. The study indicated alterations in the cytokine level, blood-brain barrier and behavior changes. Mice were exposed to penicillin from one week before birthing up until weaning began. After exposure, mice exhibited anxiety-like symptoms, impaired social behaviors and displaced aggression. The investigators also claimed that enriching the microbiome with *Lactobacillus Rhamnosus JB-1* could prevent some of the observed changes.

### 2.5.2 Review of human studies

Despite the evidence above suggesting a strong linkage between microbiome disturbance and behavior change in animal models, few studies in humans have been done to support this idea.[*] [36,179,223,278-281] In a study on the stool samples of nine extremely preterm infants, Chernikova et al. revealed that prenatal exposure to antibiotics increased the level of some pathogenic bacteria including *Staphylococcus*, *Streptococcus*, *Serratia*, and *Parabacteroides*.[278] The investigators also found that prenatal exposure decreased microbiome diversity irrespective of postnatal exposure to antibiotics. This study also showed that postnatal antibiotic exposure decreased the microbiome diversity and increased the abundance of pathogenic bacteria including *Serratia* and *Bacteroides* irrespective of prenatal exposures.

Fouhy et al. also examined the stool samples of 9 infants and showed that postnatal exposure to parenteral ampicillin and gentamicin within 48 hours of birth had decreased the levels of beneficial bacteria including *Bifidobacterium* and *Lactobacillus* even four weeks after the cessation of antibiotics.[223] These changes in infant gut microbiome persisted eight weeks after the end of treatment.

Ferrer et al. stated that a combination of ampicillin, sulbactam, and cefazolin altered host metabolome profiles of the human gut and induced changes in anabolic sugar metabolism, and the synthesis and degradation of intestinal/colonic epithelium components.[279] Consistent with previous experiments in animal models, this finding suggested that the shared host-microbiota

[*] Last update: November 25, 2018.

metabolome could be a possible source of molecules that directly or indirectly (as precursors) modify brain function.

A Canadian study examined gut microbiota profiles of 198 healthy term infants at 3 and 12 months after births.[179] This study showed that antibiotic prophylaxis given to mothers during parturition had long-lasting effects on their offspring's gut microbiome. Also, this study found a decrease in microbiome richness and an abundance of *Bacteroidetes* and *Firmicutes* including genera *Clostridium* and *Enterococcus.* These changes were specifically remarkable at the age of 3 months. They also found that breastfeeding has a protective effect against this dysbiosis. However, this study did not examine the infant meconium immediately after exposure to postpartum antibiotics. The small sample size was another limitation of this research.

In a similar vein, a European cohort examined 606 infants who were exposed to antibiotics perinatally.[280] This study analyzed the fecal samples at six weeks after birth from infants born in the UK, Germany, Spain, Sweden, and Italy. This multicenter study showed that the *Bifidobacterium* genus (40%), *Bacteroides* (11.4%) and *Enterobacteria* (7.5%) were the most prevalent bacteria in the samples. Only 7% of children received antibiotics during the first six weeks of life, and their microbiome contained 2.5 times more *Enterobacteria* than those who never received antibiotics. Children who were exposed to the maternal antibiotic in the perinatal and/ or postnatal period had a lower rate of total detectable bacteria as well as less *Bacteroides* and members of the *Atopobium* group in their fecal samples compared to non-exposed children. The findings also indicated a difference in the composition of infant gut in those who were breastfed (with more *Bifidobacteria*) compared to those who were formula fed (with more

*Bacteroides*, the *Clostridium coccoides* and *Lactobacillus* groups). Children born via cesarean also had less *Bacteroides* and members of the *Atopobium* cluster compared to those born via vaginal delivery. There was also an association between geographic origin and composition of infant gut microbiome. The major limitation of this study was the possibility of information bias since the antibiotic exposure and other risk factors were assessed based on mothers' self-report which was subject to recall bias.

Using a population-based medical record database, Lurie et al. (2015) from the United Kingdom conducted a large case-control study on 220,234 patients between the ages of 15-65 years.[281] They showed that exposure to a single antibiotic course increases the risk of depression up to 26%, anxiety up to 44% and psychosis up to 30%, and recurrent antibiotic exposure increases these risks up to 56%, 81%, and 83%, respectively. This study included a wide range of age groups from adolescents to the elderly without reporting the increased risk of depression and anxiety for each age group. The authors examined SES, smoking, alcohol consumption and previous infections as potential confounders but failed to report when the exposures started in the patients' postnatal life.

In a similar vein, Hamad et al. (2018) conducted a large population-based cohort study comprising 214, 834 children born in Manitoba, Canada.[36] They assessed the association between exposure to antibiotics in the first year of life and development of Autism Spectrum Disorders (ASD). The results did not indicate any significant association. The authors examined many confounders including sex, region, health care access, SES, maternal age at delivery, prenatal antidepressants use, gestational age, medical comorbidities, birth complications,

delivery method, multiple births, breastfeeding initiation, birth order, year and season of birth. The authors did not use a validated algorithm for ASD diagnosis and could not assess medication compliance. Even though this study did not focus on assessing mood and anxiety disorders, it is possible that the underlying mechanisms for all disorders to be the same due to the co-occurrence of anxiety and ASD.[282]

### 2.6 Conclusion

As the child population in Manitoba continues to increase, the number of children with mental illnesses like mood and anxiety disorders in this population will also continue to rise. When the population of mentally ill children grows, the costs of their health care utilization will also continue to increase. Therefore, exploring the associated risk factors will highlight the value of preventive interventions in the future. Within biological, family and school contexts, some potential risk factors identified in the literature included biological sex, SES, living place, gestational age, birth weight, infections, and comorbid chronic diseases such as diabetes, IBD, atopic dermatitis, seizure disorders, and cancer. Seasonality, vitamin D and medications were also associated with mood and anxiety disorders in the general population. A potentially modifiable risk factor which was recently identified in the literature is antibiotic exposure in early life (because of maternal or child antibiotic use) even though the underlying mechanism of mood and anxiety disorders, especially in children, is not well established.

The information obtained from the literature review suggests evidence of antibiotic-induced dysbiosis in both human and animal studies as well as the antibiotic impact on brain function in

animal models. However, no study has focused on the linkage between antibiotic exposure and the risk of developing mood and anxiety disorders in children and adolescents.

The results from two previous human studies on psychiatric disorders have been inconclusive, with limitations such as unknown exposure period, varying age groups and assessing different outcomes such as autism. The first study measured only two confounders to examine the association between exposure to antibiotics at some unknown time in postnatal life and psychiatric disorders in the general population (not specified for children population).[281] The second study examined more confounders, and the results seemed to be more reliable, but the investigators did not assess mood and anxiety disorders in children as an outcome of interest.[36] The current study will address the existing knowledge gap and limitations from previous studies by focusing on mood and anxiety disorders in a specific population (Manitoba children and adolescents) after a defined antibiotic exposure period (early life).

Last, the new information gleaned from this dissertation will supplement existing literature about the potential role of gut-brain-axis on behavior change. It will also provide a comprehensive understanding of the association between antibiotic exposure and the subsequent development of mood and anxiety disorders in children and adolescents.

# Chapter 3

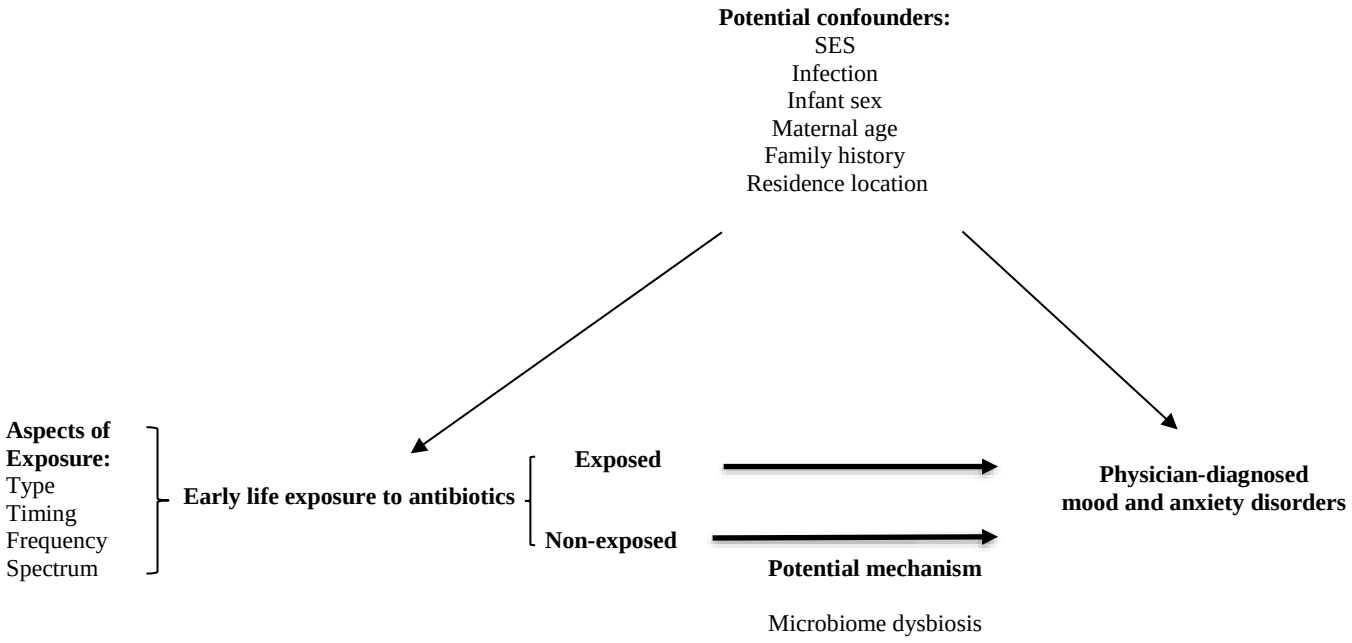
# Methods

This chapter will provide an overview of the study protocol. The four basic research elements used for choosing the current study approach will be discussed, followed by a description of the retrospective cohort design of the study, study population, datasets, variables and statistical analyses. Finally, ethical considerations will be addressed.

## 3.1 Overview of the study protocol

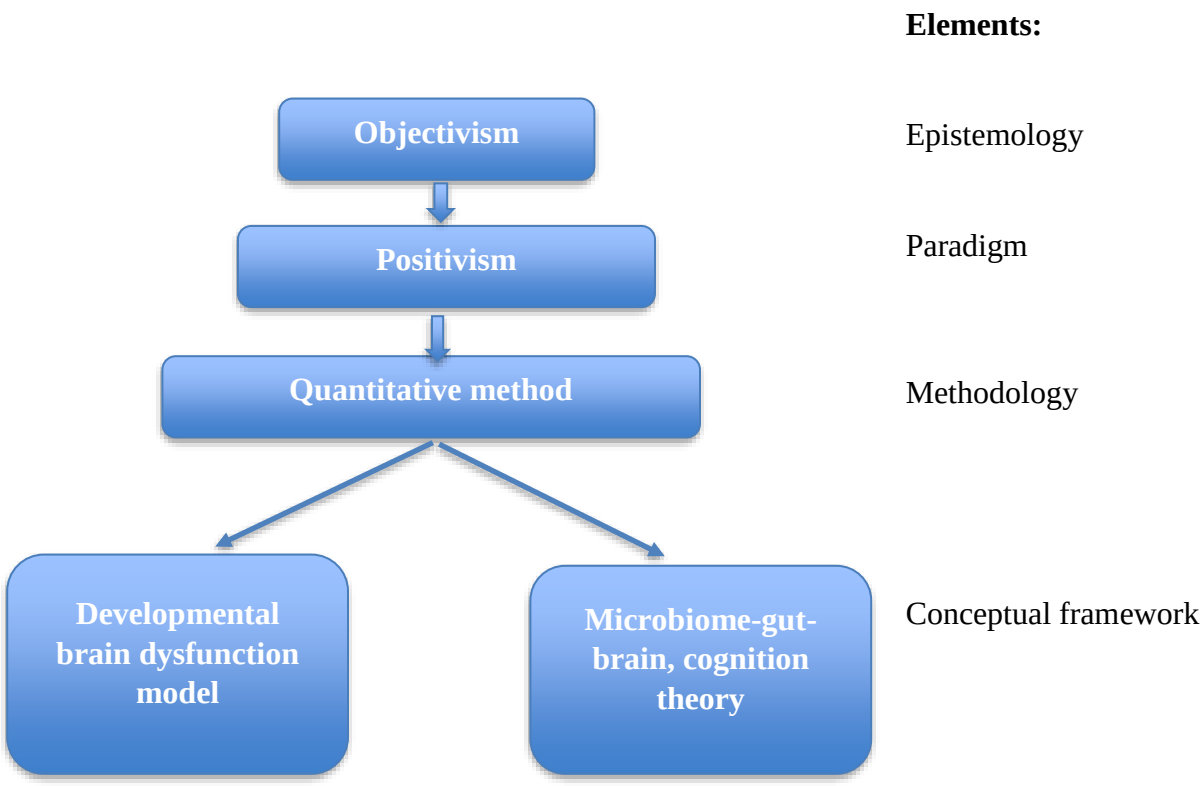
A population-based retrospective cohort design was used in this dissertation to examine the association between exposure to antibiotics and the development of mood and anxiety disorders in children and adolescents born in Manitoba, Canada between January 1, 1996, and December 31, 2012. The outcome of interest was the development of physician-diagnosed mood and anxiety disorders during childhood and adolescence. A conceptual model was created and presented in **Figure 5.** To address the four main objectives of the study, administrative data available at MCHP were used to carry out survival analysis including Cox proportional hazards models. The study objectives that were introduced in the first chapter will be reviewed later in Chapter 5. The selection of the current research approach was based on the four essential elements introduced by Crotty[283] which includes epistemology, paradigms, methodology and theoretical framework. These elements are presented in **Figure 6** and will be discussed in depth in the following section.

**Figure 5- Conceptual model outlining the potential association between antibiotic exposure in early life and the development of mood and anxiety disorders***




*The term "early life" used in this diagram indicates the fetal life (prenatal period) and the postnatal period from birth to the end of three years of age. The full list of potential confounders is presented later in this chapter.

**Figure 6 - Crotty's research design elements used in the current study**



### 3.2 Research design elements

Crotty's four research design elements that were involved in the selection of the current research approach are presented in **Figure 6**. The first step is to determine the epistemology. Epistemology refers to "the theory of knowledge embedded in the theoretical perspective and thereby in the methodology" (p. 3).[283] It is also a "way of understanding and explaining how we know what we know" (p. 3).[283] There is quite a range of epistemologies: objectivism, constructionism and subjectivism. The current study is guided by an objectivist epistemology worldview, which "holds that meaning, and therefore meaningful reality exists as such apart from the operation of any consciousness" (p. 3).[283] Objectivism comes to the fore in the context of paradigms mainly named as positivism and post-positivism.[283]

Paradigms or theoretical perspectives are the second component involved in a research approach and are defined as "the philosophical stance informing the methodology and thus providing a context for the process and grounding its logic and criteria" (p. 3).[283] This study was conducted within a positivist paradigm as positivism conveys a deterministic philosophy in which outcomes are the result of causes.[284] Problems studied within this paradigm reflect the need to identify and assess the factors which cause the outcome. Positivism informs a quantitative methodology, which governs our choice and use of methods. This approach examines the relationship among variables by measuring them using statistical procedures.[284] With a positivist approach and quantitative methodology, this study was informed by two conceptual frameworks: the developmental brain dysfunction model and the microbiome-gut-brain, cognition theory which will be discussed in the following section.

### 3.2.1 Developmental brain dysfunction theory

The developmental brain dysfunction model was proposed by Myers,[285] and later adapted by Moreno-De-Luca.[286] This model illustrates some factors that may affect the development and function of the human brain. These factors include genetic, trauma, infections, metabolites, toxins, teratogens (an agent with a harmful effect on the embryo or fetus), and hypoxia-ischemia due to low oxygen and blood supply.* As depicted in **Figure 7,** brain dysfunction can have different manifestations in cognitive, neuro-motor, neuro-behavioral or neuro-anatomical domains which underlie clinical diagnosis of various psychiatric disorders.

Environment and experiential factors can directly insult the brain, mediate the effect of the listed factors on the brain and/or modify the manifestations of brain dysfunction. Moreno-De-Luca et al. used the term "developmental brain dysfunction" and suggested that if the listed factors cause minimal dysfunction in the developing brain, less severe disorders such as learning disabilities, and attention deficit hyperactivity disorder (ADHD) will be developed otherwise severe diseases will occur. These severe disorders include cerebral palsy, autism spectrum disorders (ASD), schizophrenia and affective disorders.

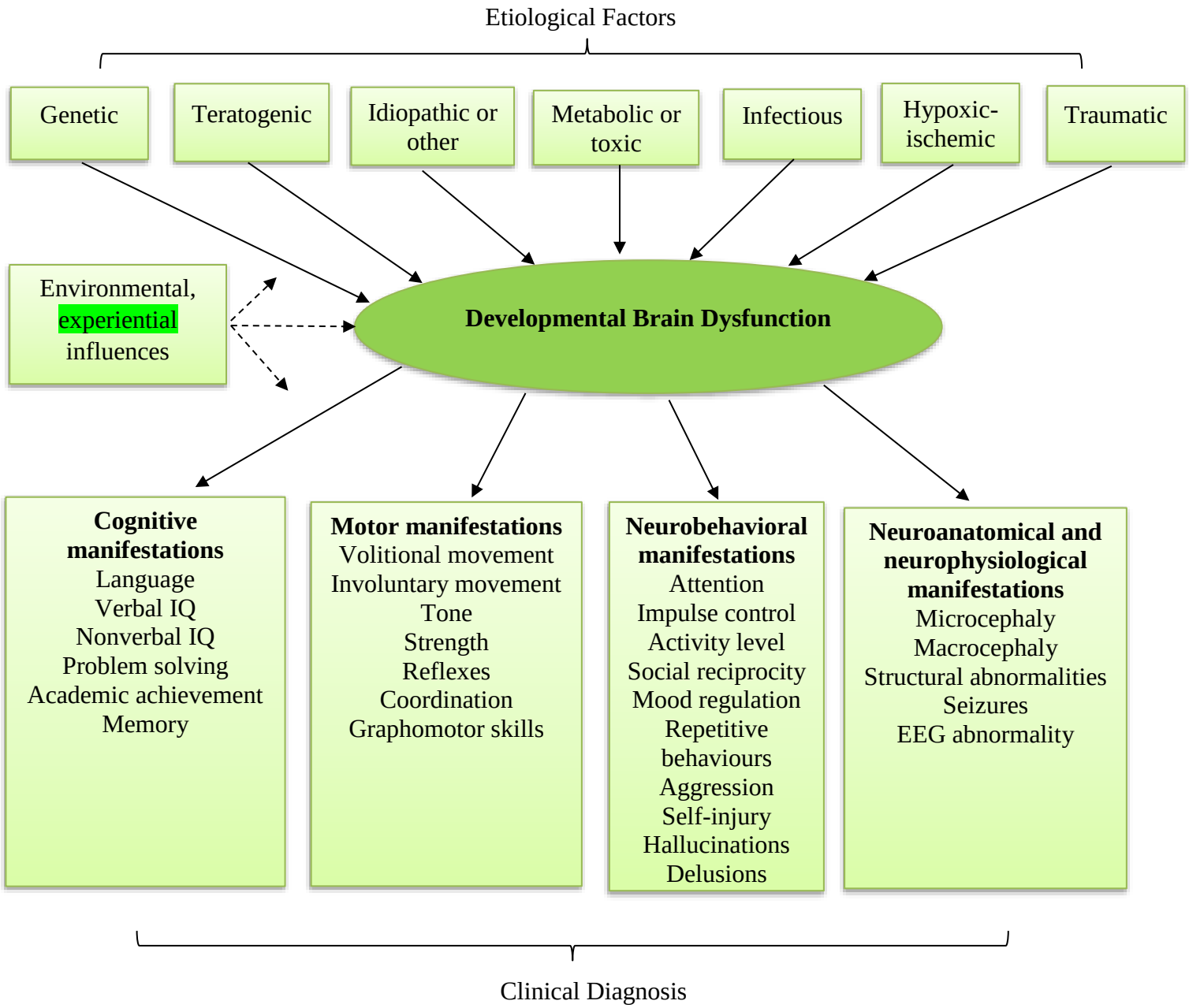
Moreno-De-Luca argues that even though neurodevelopmental and neuropsychiatric disorders may have different symptoms, but they may share similar pathophysiology and etiological factors. This notion indicates that clinically heterogeneous disorders may still be etiologically homogenous. Also, this model shows that each of the listed factors has the potential to contribute to the development of a variety of dysfunctional brain disorders. Consequently, this model

*The author did not specifically define any of these factors and did not provide any list for potential teratogens.

supports the notion of the co-occurrence of mood disorders and anxiety disorders as well as seizures, ASD, ADHD, psychotic and developmental disorders. As depicted in **Figure 7**, the symptoms of these disorders are listed in different categories.

Moreover, this model supports the idea discussed in Chapter 2, that genetic, infection and different environmental influences (e.g., medications) are likely to contribute to the development of mood and anxiety disorders. Within this conceptual framework, these factors have the potential to disrupt the normal function of the developing brain and cause neurobehavioral manifestations such as mood dysregulation. Thus, exposure to antibiotics can be integrated into this model as an environmental factor while moderating the effect of infections (as the target of treatment) on the brain. There is another possibility that both antibiotic and infections affect the brain via a common pathway which will be discussed in the second theoretical framework named microbiome-gut-brain, cognition theory.

**Figure 7- Developmental Brain Dysfunction Model***




(Copied and reproduced with permission from Moreno-De-Luca, 2013)[286]

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### 3.2.2 Microbiome-gut-brain, cognition theory

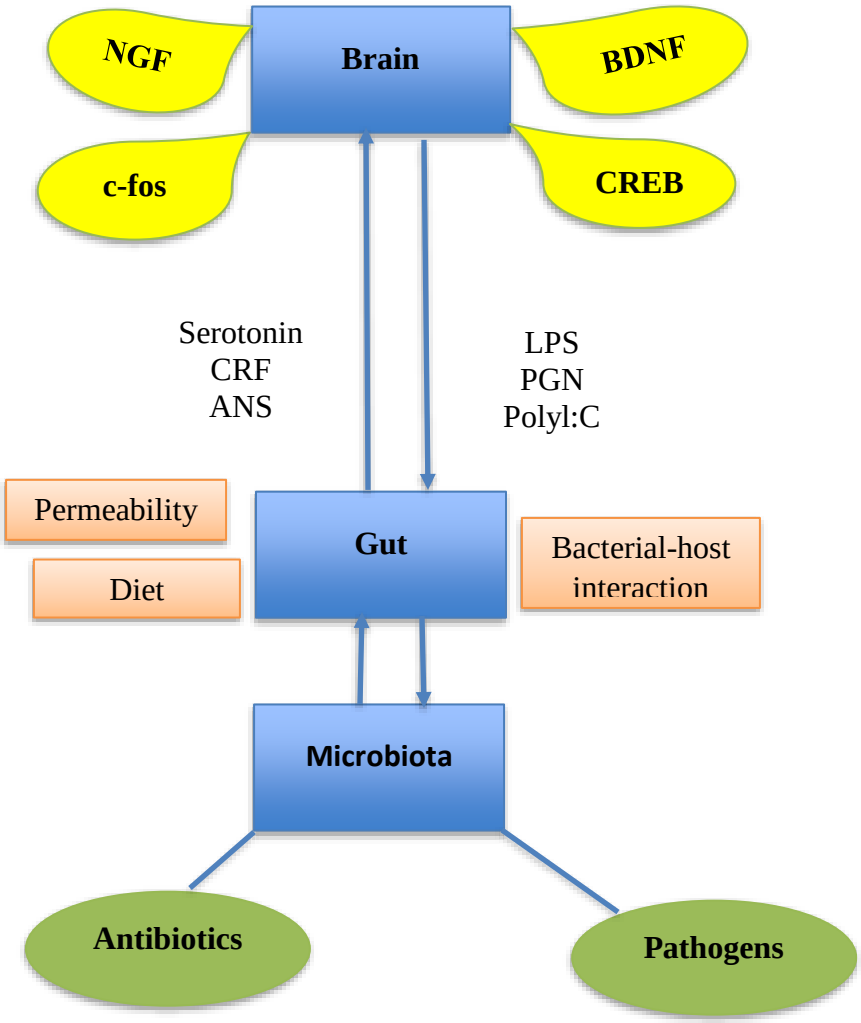
Another conceptual framework which provides a possible explanation for the impact of antibiotic exposure on the developing brain is the microbiome-gut-brain, cognitive theory which was suggested by Gareau.[149] As depicted in **Figure 8,** this animal-based model illustrates numerous factors that are involved in the bidirectional gut-brain-microbiome axis and overall changes in cognitive behavior. According to this model, both antibiotics and pathogenic bacteria can affect the microbiome composition, gut permeability and gut interactions with the pathogens. Pathogenic bacteria produce chemical components such as lipopolysaccharide (LPS), peptidoglycan (PGN)) and polyinosinic: polycytidylic acid* (poly I:C). These chemicals, specifically LPS, can induce inflammatory signaling.[287] Increased signaling[288] can alter brain function (specifically in the hippocampus) and lead to changes in the expression of some metabolites in the brain.[149] Gareau listed these metabolites as a nerve-growth factor (NGF)[149] which is involved in cognitive function;[289] brain-derived neurotrophic factor (BDNF)[149] which is involved in synaptic plasticity during neurogenesis;[290] c-fos†and cyclic adenosine monophosphate (cAMP) response-element binding protein (CREB)[149] which are involved in memory function.[291]As a result, cognitive deficits and memory dysfunction may occur.

This model was used for exploring the underlying mechanism of the effects of antibiotic exposure on the brain and its neurobehavioral manifestations. According to this model, antibiotics can operate singly or in combination with infections (as the target of treatment) to affect the brain via similar pathways. Diet and stress as confounders can also modulate these

*An immune-stimulants used to stimulate a viral infection.
†A proto-oncogenic produced after depolarization of neurons

mechanisms. Exposure to stress can stimulate the production of corticotrophin-releasing factor (CRF) which can affect the brain function either via “the autonomic nervous system (ANS) or the activation of the serotonergic system.” (p. 366).[149] Even though this model does not show any connection between bacterial infection and the source of stress, Gareau et al. in another study found that pathogenic bacteria can cause memory dysfunction in mice only after exposure to acute stress.[292]

**Figure 8- Microbiome-Gut-Brain and Cognition Theory***




(Copied and reproduced with permission from Gareau MG. 2014)[149]

NGF: nerve-growth factor; BDNF: brain-derived neurotrophic factor, c-fos:a proto-oncogene; CREB: cyclic adenosine monophosphate (cAMP) response element binding protein; ANS: autonomic nervous system; LPS: lipopolysaccharide; PGN: peptidoglycan; polyl:C: Polyinosinic: polycytidylic acid

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### 3.3 Study design

A cohort study design was used in this dissertation to compare differences in the incidence of the outcome of interest between antibiotic exposed children and their unexposed counterparts. This study design is an ideal approach in observational pharmacoepidemiologic studies when the individuals have entered the cohort based on the presence or absence of exposure, and their subsequent development of a disease is assessed.[293,294] In comparison to case-control studies, this design is free of the problems for selecting a control group and the observed associations are more likely to be causal.[293] A retrospective approach was also chosen for this dissertation as the data on both exposures to antibiotics and diagnostic codes for mood and anxiety disorders had been measured in the past (available in MCHP databases). Compared to prospective cohort design, this approach is also less expensive and time-consuming as it does not need any follow-up in the future which may take years to complete.[293]

Moreover, this research is a population-based retrospective cohort study using linked clinical and administrative health care databases available at the MCHP, University of Manitoba. Administrative databases are known as efficient sources which provide measures and complete follow-up for both exposure and outcome with minimal risk of recall bias.[58,295,296] The use of population-based administrative data does not rely on the recollection of mothers or their children for receiving antibiotics or on correctly stating whether or not they had been diagnosed with mood and anxiety disorders.

Moreover, MCHP has administrative data on the entire Manitoba population with information on antibiotic prescriptions (as exposure) and physician-diagnosed mood and anxiety disorders (as

outcomes) which go back for 48 years (since 1970).[297] The use of the whole-population administrative data provided the researcher with a robust, cost-effective and time-efficient resource to address the study objectives. The use of administrative data also enabled the researcher to follow study participants from pre-conception to 19 years of age with full access to all prescriptions dispensed to a mother and her child. In addition, MCHP provided the researcher access to data on all study participants irrespective of their exposure status to prescription antibiotics. Therefore, the researcher chose a population-based retrospective cohort design to take advantage of these valuable features of administrative data.

### 3.4 Datasets

The provincial administrative health system collects information on any contact that a patient has with a publicly funded facility, as well as medical experts or general practitioners who bill the provincial health plan of the patient.[298] It does not cover contacts with the medical specialists who practice in a private setting.[298]

Using Manitoba's administrative health data, an already created provincial birth cohort was used. This cohort comprised all children born in Manitoba between January 1, 1996, and December 31, 2012, who were continuously registered for at least three years after their birth and their mothers were continuously registered for at least one year before and at least one year after childbirth (N=221,139 linked dyads from 122,030 different mothers). Using the Personal Health Identification Number (PHIN) available at the hospital discharge abstracts, mothers and their children (born in Manitoba) were linked together. All the datasets were linked using scrambled PHIN code as unique encoded identifiers to maintain privacy and confidentiality of the

individual records. The de-identification process occurred before transferring data from governmental administrative datasets to Manitoba Population Research Data Repository housed at MCHP.[299] The Repository is a "comprehensive collection of the administrative, registry, survey and other data" obtained from residents of Manitoba.[299] The data were updated on March 31, 2017. Main data sources used in this dissertation were physician billing claims, hospitalization discharge abstracts, and drug prescriptions records which were collected by reliable and valid databases,[300] that are described below.

1-Manitoba Health Insurance Registry (MHIR) or Population Registry: This dataset provides demographic information such as birth date (age), place (residential postal codes) and biological sex for every single individual residing in Manitoba; i.e., all people who were registered to receive health services in Manitoba at some time since 1970.[301] The only excluded individuals are those who are insured federally (military personnel and federal inmates) or not eligible for coverage. This file also contains data fields on the dates that residents have come into or left the province and helps identify all eligible women that have undertaken maternity care in Manitoba. In this dataset, every individual has a scrambled PHIN. In 1984, Manitoba's provincial government started assigning PHIN for each resident at birth or after moving into Manitoba.[301] This dataset was used to obtain coverage over time, place of residence, birth dates, mortality and the number of children in a household.

2-Physician claims or medical claims/services: This dataset contains claims for physician visits at office, hospital and outpatient departments, and three-digit international classification of

disease (ICD) diagnosis associated with the physician visit.[302] This file was used for the identification of outcome and co-morbidities (diagnosis codes).

3- Drug Program Information Network (DPIN). DPIN is "a point-of-sale outpatient prescription drug database" which contains drug profiles for all Manitoba residents regardless of their insurance coverage (p. 1440).[302] The indication of prescription, hospital pharmacies, nursing stations, ward stock and outpatient's visits at Cancer Care Manitoba are not included in this file. This dataset captures data on drugs dispensed (not drugs that were taken). DPIN was used to track any antibiotic prescription for infection during pregnancy and early childhood as well as any medications for mental health disorders and comorbidities.

4- Hospital Discharge Abstract Database (HDAD). The HDAD is a collection of "hospital records containing summaries of demographic and clinical information" that are abstracted at the time of discharge and includes diagnostic codes, sex and postal codes (p. 1440).[302] HDAD was used to track a mother or child's admission to hospitals due to mental health disorders or comorbidities. In addition to the identification of outcome (diagnosis codes), birth events were assessed using HDAD. This database does not have information about prescribed medications.

5- Diabetes Education Resource for Children and Adolescents (DER-CA): This is a clinical health dataset containing information on children diagnosed with diabetes in Manitoba since January 1986.[303] This dataset was used for the diagnosis of diabetes (as comorbidity) in children.

6- Canada Census: This dataset is based on a population survey conducted every five years and contains information at area level for each person within a dissemination area in Canada. This dataset was used to measure SES based on socioeconomic factor index - version 2 (SEFI-2) and income quintiles in the following years:

1996 census: 1994-1998 income quintiles

2001 census: 1999-2003 income quintiles

2006 census: 2004-2008 income quintiles

2011 census: 2009-2013 income quintiles

All the major databases that the investigator had access through MCHP have been outlined and presented in **Table 2.**

**Table 2. Databases accessed through MCHP and relevant information extracted**

| Variables | Database and date accessed | Information retrieved |
|---|---|---|
| Demographics | MHIR (1970-2012) | Birthdate (age), the season of birth, place of residence (using the postal code and municipal code), mortality, number of children, and coverage benefits of the study population. |
| | Canada Census (1996-2011) | Income quintiles, SEFI-2 |
| | MHIR (1970-2012)<br>HDAD (1994-2017) | Maternal age at the birth of cohort child and age at the birth of the first child |
| | HDAD (1994-2017) | Gestational age, birth weight, multiple births, delivery method, infant sex, breastfeeding initiation |
| Mood and anxiety disorders,<br>Maternal history of mental health disorders | HDAD & Medical Services (1994-2017) | ICD diagnostic codes |
| | DPIN (1994-2017) | Prescription dispensations |
| Antibiotic exposure | DPIN (1994-2017) | Prescription dispensations |
| Medical comorbidities | HDAD & Medical Services (1994 -2017) | ICD diagnostic codes |
| | DPIN (1994-2017) | Prescription dispensations |
| | DER-CA (1996-2017) | Child diabetes |
| Health care utilization | HDAD (1994-2017) | Hospital discharge |
| | Medical Services (1994-2017) | Physician visit |

### 3.5 Study sample

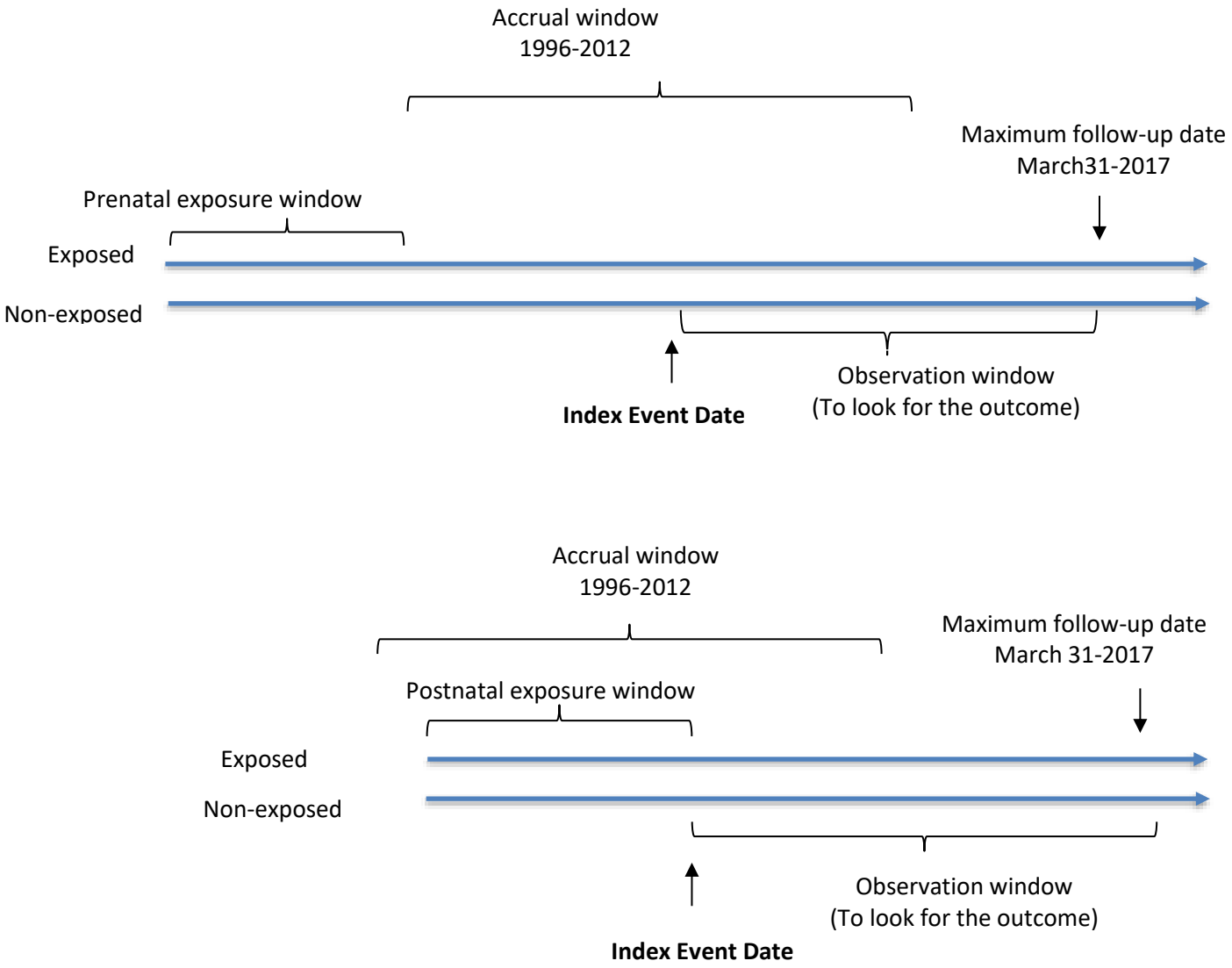
The study sample used in this dissertation included all mothers and their children who were born in the province of Manitoba, Canada between January 1, 1996, to December 31, 2012. This accrual period was chosen based on data availability. The investigator had access only to the data from these dates.

#### 3.5.1 Inclusion criteria

In this study, the WHO classification[304] was used to define a child as any Manitoba resident aged 0-19 inclusively. For descriptive purposes, two different age ranges were also created: age less than ten years as early childhood, and age between 10 to19 years as late childhood or adolescence. After identifying children in the appropriate age range (born in 1996-2012 with a maximum age of 19 years old), the study sample was restricted to those children who have Manitoba health coverage with a minimum of 3 years from birth and their mothers have had health coverage for at least one year before pregnancy and at least one year after childbirth. These periods were chosen to ensure that adequate information for antibiotic prescriptions was available for all included dyads in this study.

The index event date was the third birthdate of the child. After cohort entry, children's antibiotic prescriptions from birth to the index date as well as their mother's prescriptions during pregnancy were recorded. Accordingly, two cohorts were created using the same study population. As presented in **Figure 9**, a cohort was created to assess maternal antibiotic prescriptions during pregnancy (prenatal cohort) and a second cohort was created to identify child antibiotic prescriptions before the index date (postnatal cohort).

**Figure 9 - Timeframe for prenatal and postnatal cohorts**



### 3.5.2 Exclusion criteria

The only exclusion criterion was any record with invalid or missing data for the variables that needed to be assessed and adjusted. The study sample was already restricted to children who were born alive and experienced their third birthdate as Manitoban residents. Therefore, stillbirths and children whose health coverage was ended before the 3rd birthdate (because of either death or migration) were already excluded from the initial cohort. This exclusion was considered to ensure survival to age three years to allow for equal opportunity for exposure which would minimize survivor treatment selection bias.[305] After that point, the researcher focused on the outcomes of interest.

## 3.6 Variables

### 3.6.1 Exposure definition

The exposure variable was antibiotic systemic use in pregnant women and children under the age of three years. While antibiotic use cannot be captured within MCHP data, drug prescription was identified as a core indicator of drug use.[306] Accordingly, the antibiotic prescription was determined from community pharmacy dispensed prescriptions (DPIN) available within the MCHP. After determining the antibiotic prescription from dispensing records, it was classified by dose, timing, class and spectrum. These aspects of exposure were assessed as research has already shown that age at exposure, type and severity of exposure are significant predictors of future psychopathology, and are critical factors in mediating the impact of prenatal programming on mental health outcomes.[307,308] For this study, two types of exposure were assessed: prenatal and postnatal.

#### 3.6.1.1 Prenatal exposure

Using maternal prescription dispensations and following the ATC classification system, prenatal exposure* was defined as having one or more maternal prescriptions for any antibiotic (ATC J01) dispensed during pregnancy.[309] Maternal antibiotic prescription was classified by dose (number of prescribed courses), timing (trimesters of pregnancy), the spectrum of antibiotics (narrow vs broad) and type of antibiotic (J01A to J01X). Maternal antibiotic prescriptions within nine months before and nine months after pregnancy were also recorded for additional analyses.

#### 3.6.1.2 Postnatal exposure

Using child prescription records and following the ATC classification system, postnatal exposure was defined as having one or more child prescriptions for any antibiotic (ATC J01) dispensed during the first three years of life.[36] Child antibiotic use was classified by dose (number of prescribed courses), timing (the first year versus the second year versus the third year), spectrum of antibiotics (narrow versus broad) and type of antibiotic (J01A to J01X).

### 3.6.2 Outcome measurement

#### 3.6.2.1 Mood and anxiety disorders algorithm

Following an accepted and previously used algorithm which was introduced by Chartier et al.,[12] codes from medical services, HDAD and DPIN were used to define physician-diagnosed mood and anxiety disorders after three years of age. Accordingly, a child was identified with mood and anxiety disorders when he/she met any of the following criteria: "1) At least one hospitalization

*This was determined using a varible (prvddt) in DPIN indicating the date of the prescription that was dispensed at the pharmacy, i.e., date provided. If the date of filled prescription was between the date of conception and the child birthdate, it was assumed that the mother had received that antibiotic.

record with any of the following diagnoses: depressive disorder, affective psychoses, neurotic depression, adjustment reaction, anxiety state, phobic disorders, obsessive-compulsive disorders; 2) At least one hospitalization with a diagnosis of anxiety disorders and one or more prescriptions for an antidepressant or mood stabilizer; 3) At least one physician visit with a diagnosis of a depressive disorder or affective psychoses; 4) At least one physician visit with a diagnosis of anxiety disorders and one or more prescriptions for an antidepressant or mood stabilizer; 5) Three or more physician visits with a diagnosis of anxiety disorders or adjustment reaction" (p.44).[12] The full description of codes related to this algorithm is presented in **Appendix 3. ≥**

#### 3.6.2.2 Outcome codes for administrative data

Hospitalization records were collected from HDAD where DIAG1-25 variables were searched for relevant entries. The relevant ICD-10 codes were bipolar affective disorder (F31), depressive episode (F32), recurrent depressive disorder (F33), dysthymia (F34.1), other single mood or affective disorders (F38.0), other recurrent mood or affective disorders (F38.1), phobic anxiety disorders (F40), social phobias (F41.0), generalized anxiety disorder (F41.1), mixed anxiety and depressive disorder (F41.2), other mixed anxiety disorders (F41.3), other specified anxiety disorders (F41.8), anxiety disorder, unspecified (F41.9), obsessive-compulsive disorder (F42), post-traumatic stress disorder (F43.1), adjustment disorders (F43.2), other reactions to severe stress (F43.8), separation anxiety disorder of childhood (F93.0), other anxiety disorders (F41), obsessive-compulsive disorder (F42), dissociative disorders (F44), somatization disorder (F45.0), undifferentiated somatoform disorder (F45.1), hypochondriacal disorder (F45.2), other neurotic

disorders (F48), elaboration of physical symptoms for psychological reasons (F68.0), and mental health disorder that was not otherwise specified (F99).

The relevant ICD-9 codes included: recurrent manic disorder (296.1), depressive psychosis (296.2), recurrent depressive psychosis (296.3), bipolar affective disorder-manic (296.4), bipolar affective disorder-depressive (296.5), bipolar affective disorder not specified (296.7), manic-depressive (296.8), neurotic disorders (300), anxiety state (300.0), phobia (300.2), obsessive compulsive (300.3), neurotic depression (300.4), hypochondriasis (300.7), adjustment reaction (309), depressive disorder not elsewhere classified (311).

Physician claim records were also gathered from the medical services database where the ICD-9 diagnostic codes were reported. The relevant ICD-9 codes included affective psychoses (296), neurotic disorders (300), adjustment reaction (309) and depressive disorders which were not elsewhere classified (311).

The last potential indicator for the diagnosis of mood and anxiety disorders was a drug-filled prescription in the DPIN database for an antidepressant or mood stabilizer using the following ATC codes: N05AN01 (lithium), N05BA (benzodiazepines derivatives), N06A (antidepressants), N05BE01 (buspirone).

#### 3.6.2.3 Validation of mood and anxiety disorders algorithm

The criteria used in this algorithm were based on ICD-9-Clinical Modification (CM) and ICD-10-Canadian (CA) diagnostic codes of mood and anxiety disorders which were used by

physicians, psychiatrists, pediatricians, and other related specialists. Chartier et al. discussed the criteria with psychiatrists and reported that their algorithm was valid as it could define what it was supposed to measure (face validity). However, they did not compare the criteria with a chart review or a clinical database as a reference standard. Accordingly, they could not determine the accuracy (sensitivity and specificity) of their algorithm. Other definitions of mood and anxiety disorders used in previous reports at MCHP also lacked accuracy measurements.[310-312]

### 3.6.3 Potential confounders

Confounders are variables with independent relationship to the drug exposure and outcome of interest which can distort the true estimate effect of an association.[293,313] Accordingly, several factors associated with the development of mood and anxiety disorders were considered as potential confounders in this study. Detailed information on these risk factors was outlined in Chapter 2. The following *a priori* covariates documented from administrative health records were assessed.

#### 3.6.3.1 Demographic variables

The following baseline characteristics of the exposed and non-exposed groups were explored at the time of childbirth and were compared for identifying potential confounding variables.

1- Infant sex: The biological sex of the cohort child as a categorical variable was coded as either male (0) or female (1) and was reported in the MHIR.[314] As discussed in Chapter 2, this variable is known as a potential risk factor for the development of mood and anxiety disorders in children.

2- Gestational age or weeks after fertilization/conception,[315] was measured as a continuous variable and was retrieved from HDAD. As discussed in Chapter 2, this variable is known as a potential risk factor for the development of mood and anxiety disorders in children.

3- Birth weight as a continuous variable was defined as the weight of a newborn recorded at birth and measured in grams.[316] It was retrieved from HDAD. As discussed in Chapter 2, this variable is known as a potential risk factor for the development of mood and anxiety disorders in children.

4- Delivery method was measured as a binary variable (vaginal delivery or cesarean birth) and was retrieved from HDAD. The delivery method is not directly associated with childhood or adolescent psychological well-being,[317] but as discussed in Chapter 2, it is identified as an influential factor in the establishment of microbiota and its developmental variations which in turn may be linked to the development mood and anxiety disorders.

5- Number of births per pregnancy was measured as a binary variable to indicate if the child of the cohort was the result of a single birth (code=0) or multiple births (code=1) defined as "the birth of two or more children during a single delivery with a gestation of 20 weeks or more."[318] This variable was retrieved from HDAD. As discussed in Chapter 2, the literature supporting any association of multiple births with mood and anxiety disorder in offspring is lacking. However, the researcher measured this factor for the first time in this context

6- Infant feeding status at hospital discharge was assessed as a categorical variable based on the information whether breastfeeding during the hospital stay had been initiated (code=1) or not (code=0)[319]. Breastfeeding initiation was measured when a mother started feeding her newborn on milk from her breast.[320] The information for this covariate was retrieved from HDAD. As discussed in Chapter 2, the evidence supporting the role of breastfeeding in the development of childhood mood and anxiety disorders is lacking, but the investigator decided to examine it since it is associated with infant microbiome development which in turn may be linked to mood and anxiety disorders.

7- Season of birth was measured as a categorical variable based on the month of birth. Using the meteorological definition,[321] each season begins on the first day of the month and was classified as spring (March 1 to May 31); summer (June 1 to August 31); fall ( September 1 to November 30); and winter (December 1 to February 28 /February 29 in a leap year). As discussed in Chapter 2, this variable is known as a potential risk factor for the development of mood and anxiety disorders in the general population with no evidence in children. However, the investigator decided to measure this factor for the first time in the context of childhood mood and anxiety disorders.

8- Location of residence was measured as a categorical variable indicating either urban (1= Winnipeg or Brandon) or rural (0= other areas in Manitoba) and was reported in the MHIR. As discussed in Chapter 2, this variable is known as a potential risk factor for the development of anxiety disorders in children.

9- Socioeconomic status (SES) was measured as both categorical and continues variables using Canada census which has created income quintiles and SEFI-2. Income quintile is known as an area-level income measure ranging from 1st quintile (Q1 as the lowest) to 5th quintile (Q5 as the highest) income level.[322] As an indicator of non-medical social determinants of health,[323] SEFI-2 was first introduced by Metge et al. in 2009.[324] SEFI-2 indexes include the unemployment rate at age 15 and above, average household income at age 15 and over, the proportion of adults at age 15 and over without high school education and the proportion of single-parent families. SEFI2 scores less than zero indicates a more favorable SES than SEFI-2 scores greater than zero.[325] As discussed in Chapter 2, SES is known as a potential risk factor for the development of mood and anxiety disorders in children.

10- Maternal age at the birth of index child and maternal age at the birth of first child, live or dead, was measured as both continuous and categorical variables by grouping it into one of the three categories: less than 20, 20-35 and more than 35. The reference group for statistical testing was the 20-35 age group reflecting the normal reproductive age period.[326] Maternal age was assessed based on the date of the mother's birth reported in the MHIR and data of childbirth reported in HDAD. As discussed in Chapter 2, there is not sufficient evidence in literature supporting the role of maternal age in the development of mood and anxiety disorder in children. However the mother's age at first birth, particularly, has been identified as a strong predictor of various (positive and negative) health outcomes in children.[135,327] The influential positive role of this factor could be explained by the association with increased participation of younger mothers in the prenatal care,[135,328] likelihood of breastfeeding and child immunization.[327] A study showed that approximately 50% of the Manitoban mothers who were receiving prenatal care and

benefits, had their first babies during their teenage years.[135] However, the adverse health outcomes related to young maternal age, may be due to the biological immaturity and sociodemographic disadvantage associated with parenting in younger ages.[329,330] The literature supporting any association of maternal age at first birth with mood and anxiety disorder in offspring is lacking. However, the researcher measured this factor for the first time in this context.

11- Household number/family size is defined as the number of children aged 0-19 years living together in one household including the cohort child.[331] This variable was measured as both numerical and categorical variables by grouping it into one of the four categories (1,2,3, ≥4). The information for this variable was retrieved from the MHIR. Family size has been linked inversely to child well-being and education.[332] This negative association may be explained by resource dilution and a lower level of average maturity in the household.[332] No specific study has examined this factor in the context of mood and anxiety disorders. The investigator decided to measure this factor for the first time.

#### 3.6.3.2 Health care utilization

This variable was defined as "the measure of the population's use of the health care services available to them,"[333] assessed based on physician visits as any ambulatory contact reported in medical service dataset and hospital discharge obtained from HDAD. History of child and maternal health care utilization was recorded before the event date and was measured as both numerical (total number of visits/ hospitalizations) and categorical variables (1= any history of a physician visit or hospitalization, 0= no history). This variable was measured because there is

evidence suggesting a relationship between depression and health care utilization in individuals affected by chronic diseases.[334] McRae et al. also reported that Canadians who received health care services for mood and anxiety disorders were more often diagnosed with chronic diseases such as asthma and diabetes compared to Canadian who did not receive such services.[18] It seems that the utilization of hospital and physician resources is associated with more diagnoses of both mood and anxiety disorders and comorbidities. Accordingly, the investigator assessed this variable to address confounding by indication.

#### 3.6.3.3 Maternal history of mental health disorders

Maternal history of mental health disorders including mood and anxiety disorders, ADHD, developmental disorders, ASD, psychotic disorders, schizophrenia, conduct disorders, suicide attempt and substance use disorders using the algorithm described in **Appendix 3**, were assessed based on information available at DPIN, medical services and HDAD within five years preceding the index date. Each maternal mental illness was separately coded (1= diagnosis, 0= no diagnosis). As discussed in Chapter 2, the possibility of developing mood and anxiety disorders is higher when a relative has the same disorder, but there is also evidence indicating that children may be at risk of developing a mental illness other than the one that their parents suffer.[335] Accordingly, the investigator decided to examine some maternal mental health disorders that could be captured by administrative databases.

#### 3.6.3.4 Child medical comorbidities

As explained in Chapter 2, some chronic diseases are comorbid with mood and anxiety disorders. The comorbidities including asthma, diabetes, IBD, atopic dermatitis, seizure disorders,

hypothyroidism, and cancer were assessed using the algorithm described in **Appendix 3**. Each comorbidity was separately coded (1= diagnosis, 0= no diagnosis) and assessed at the event date using relevant diagnoses from hospitalizations and outpatient visits as well as prescriptions which were identified in the HDAD, medical services and DPIN.

### 3.7 Statistical analyses

Before the onset of the study, the researcher set *a priori* significance level of $p < 0.05$. To examine the association between antibiotic exposure and the development of mood and anxiety disorders, the following steps were taken.

#### 3.7.1 Data management

As a first step in the examination of the research questions being asked, the related datasets were first identified, and their contents were verified running contents procedures in statistical analysis system (SAS) programming version 9.4. Variables of interest that were not available in the datasets were created (indicators) using previously identified algorithms. Next, the missing and inaccurate values for all variables of interest were checked prior to further analyses using frequency procedures. The datasets were finally cleaned and concatenated into one dataset for further analyses. Next, descriptive and univariate statistics of each variable within the new dataset were explored.

#### 3.7.2 Comparing baseline characteristics between exposed and non-exposed groups

After determining the distribution of each variable using Kolmogorov-Smirnov (KS) test, the baseline characteristics of children exposed to antibiotics were compared with their non-exposed

counterparts to identify any imbalanced covariates that may act as a confounder. To indicate significant covariate imbalance, a standardized difference (Std. Diff.) of 0.1 or higher was used for comparison.[336] This indicator was chosen because the large sample size of the study may lead to statistically significant differences between groups while they are not clinically relevant.[293] The investigator compared categorical covariates using the Chi-square ($\chi 2$) test of independence to determine differences in proportions. Since all variables were not normally distributed, the Mann-Whitney U test was used to compare the means of continuous variables between groups.

To obtain an unbiased estimate of the effect size, the imbalanced covariates in exposed and non-exposed groups, as well as previously reported predictors in the literature for mood and anxiety disorders, were stratified using Cochran-Mantel-Haenszel Statistics.[337] If the results from the Mantel-Haenszel test showed significant differences in the risk of developing mood and anxiety disorders at the different level of stratified variables, that covariate was considered as a potential confounder for further analyses.

### 3.7.3 Checking for multicollinearity

The next step in examining an association was to check for multicollinearity among covariates because the overlapping information could lead to misleading interpretations.[338] After identifying those covariates that were statistically and/or conceptually (based on the literature review) associated with exposure and/or outcome (potential confounders), multicollinearity among variables was checked using the following complementary approaches.

First, the correlation matrix was explored through the implementation of the correlation procedure in SAS. A correlation coefficient of 0.5 or higher was considered as an indicator of collinearity.[338] Upon reviewing the correlation matrix, the highest correlation coefficient in this study was identified as 0.45 which indicated no apparent collinearity among target variables.

Second, a regression procedure was performed for examining multicollinearity through the variance inflation factor (VIF) and tolerance. Any value below 0.1 for tolerance and any value above 10 for VIF were considered as an indicator of multicollinearity.[338,339] No threat of multicollinearity was identified through the tolerance analysis as the lowest value for selected covariates was 0.5676. The highest value of VIF sat at 1.7617 which confirmed a lack of multicollinearity.

The third approach was reviewing the collinearity diagnostics through an eigensystem analysis of covariance comparison. Small eigenvalues (close to zero) with a corresponding large condition index was considered as an indication of multicollinearity.[340] According to the results, none of the eigenvalues and their corresponding condition indexes matched the above description. Finally, no multicollinearity was detected.

### 3.7.4 Time-to-event analysis of outcome

To address the research objectives, the investigator should know both whether and when mood and anxiety disorders took place. Therefore, it was necessary to analyze data that corresponded to the time from a well-defined time of origin until the occurrence of mood and anxiety disorders. At the time that the data was obtained, it was possible that mood and anxiety disorders

had not yet developed or were not diagnosed for certain participants. In this circumstance, conventional statistics were invalid to deal with incomplete data (censoring). Instead, survival analysis (time to event analysis), which examines the occurrence and timing of events,[341] was used to measure physician-diagnosed mood and anxiety disorders. This outcome was identified as the event of interest in this study for time-to-event analyses using Cox proportional hazard regression models. The index date was the third birthdate of the child and the follow-up period ended on March 31, 2017. Any child who reached the end of the study period without experiencing the outcome of interest was treated as a censored case for time-to-event analyses. Accordingly, children who experienced their 19$^{th}$ birthday or reached the end of the study period or their health coverage ended sooner (due to death or migration) before being diagnosed with mood and anxiety disorders, were considered as censored observations. If a child was lost to follow up due to moving out of the province, then that child was censored at the last date of his/her Manitoba health care coverage. Another censoring event was child death using the cancellation date (canccode) obtained from MHIR.

#### 3.7.4.1 Cox proportional hazards models

Cox proportional hazards model is a distribution-free model that handles ties and time-dependent covariates properly.[341] As a time-to-event analysis, Cox proportional hazards model is an appropriate analysis where the study groups have different follow-up times since it accounts for variable follow-up times between groups in its calculations.[342] Therefore Cox proportional hazards model was suited for analyses of this dissertation as the entry date to cohort varies among participants. Accordingly, Cox modelling technique was used to compute crude and adjusted hazard ratios (aHR) with 95% confidence intervals (CI) to assess the association

between antibiotic exposure and development of mood and anxiety disorders. To reduce any potential bias that could distort the true HR estimation, the following assumptions were considered.

The primary assumption of the Cox regression model known as the proportionality hazard (PH) assumption indicates that the ratio of hazard between exposed and non-exposed groups is constant over time.[341] Violation of this assumption is an indicator of the existence of an interaction between time and one or more variables. This assumption was assessed using two diagnostic procedures. In the first approach, the PH assumption was assessed by examining the correlation between follow-up time and Schoenfeld residuals of covariates. Using this method, PH assumption was not violated since the lowest values for Schoenfeld residuals with time (*p*-value= 0.5796), logarithm of time (*p*-value= 0.6397) and square of time (*p*-value= 0.4331), were all more than 0.05.

The second method involved interaction testing in which a new covariate representing the interaction of the original covariate and logarithm of time was created and added to the model. A significant coefficient for an interaction covariate indicates that the PH assumption is violated for that variable. In the evidence of any non-proportionality, the interaction covariate remains in the final modelling; otherwise, it would be dropped.[341] Using this method also showed that the PH assumption was not violated as the lowest *p*-value for the interaction terms sat at 0.0901.

Another critical assumption is to ensure that censoring events have non-informative nature indicating that the reason for dropping out of the study is not associated with the outcome. The

major censoring event in this study was the loss to follow-up due to death or migration. The cause of death was not captured in MHIR. Irrespective of the real cause of death that could be due to suicide, complication, or a previous medical condition, anyone who died before receiving a mood and anxiety disorders diagnosis may be a representative of a sicker underlying subpopulation of the selected cohort. If this subpopulation had developed mood and anxiety disorders early on or before death, the results might be distorted. To address the second assumption, all children who died after the age of 3 (within the study period) were eliminated for an additional sensitivity analysis.

Migration also seems to be independent of the study outcome because children usually live with their parents or guardians, and any movement in or out of the country or province depends on their parents' decisions which are not considered to be related to mood and anxiety disorders or its severity and therefore would not likely violate the non-informative assumption. However, an additional sensitivity analysis was performed to remove all children who were lost to follow up.

The origin time for these analyses was the third birthdate of the child which would be the day after the end of the exposure period. This date was redefined as time zero to eliminate the source of immortal time bias.[343] This was also beneficial for Cox regression analysis since a time-fixed approach was used to define the exposure.[343,344]

The outcome of interest was physician-diagnosed mood and anxiety disorders. Based on the evidence in the literature, it was expected that children could develop these disorders as young as age three.[345]

Since the assessment of multiple associations increases the likelihood of type I error, the *p*-value was also corrected by using the Bonferroni method in final modelling.[346]

### 3.7.4.2 Additional analyses

As earlier discussed, timing, type and severity of exposure are significant predictors of mental health disorders. The number of antibiotic courses was used as an indicator of the severity of exposure. Class and spectrum of antibiotics reflect the type of exposure. Accordingly, additional analyses were conducted on the primary outcome classified by different aspects of exposure to address objective two and four of this dissertation:

1-Classes of antibiotics: Using ATC codes and classifications proposed by Kapoor et al.,[182] Loewen et al.[35]and Hamad et al.,[36] antibiotics were grouped based on their most similarity in mechanism of action:

1-1-β-lactam, penicillin (J01C)

1-2-Other β-lactams (J01D)

1-3-Sulfonamides and trimethoprim (J01E)

1-4-Macrolides, lincosamides, streptogramins (J01F)

1-5-Tetracyclines, aminoglycosides, quinolones and others (J01A, J01G, J01B, J01M, J01R, J01X)

2-Spectrum: narrow or broad

3- Timing of exposure

*3-1-Prenatal exposure*: antibiotics dispensed to the mother at any time from conception* until the birthdate of the cohort child and in specific time windows:

3-1-1-First trimester: from conception to 91 days of pregnancy

3-1-2-Second trimester: from 92 days to 189 days of pregnancy

3-1-3-Third trimester: from 190 days of pregnancy to child birthdate

3-2-*Postnatal exposure*: antibiotics dispensed to the cohort child at any time from birth to three years of age and in specific time windows:

3-2-1- First year of life: from birth to 360 days of age

3-2-2- Second year of life: from 361 to 720 days of age

3-2-3- Third year of life: From 721 to 1080 days of age

4-Frequency of exposure: measured both as numerical and categorical variable (0, 1, 2, 3+).

Using DPIN, refills were counted irrespective of the prescribed daily dosage, quantity, or the number of day's supply. Overlap or gap between prescriptions are known sources of bias when measuring adherence to a single drug, and ignoring or accounting for them can lead to different estimates of exposure duration.[347,348] This study was not supposed to measure adherence to antibiotics (e.g. medication possession rate) or exposure duration; therefore it did not account for accumulated surplus (overlaps), gaps, changes in dosage, therapeutic switching and augmentation (duplication).[347] As well, this study did not measure drug interactions.

In addition to prenatal and postnatal periods as the main timing frames of exposures, the time surrounding pregnancy as pre-pregnancy and post-pregnancy exposures was also outlined to be assessed when planning the dissertation proposal. Accordingly, pre-pregnancy exposure was

*The date of conception was calculated by subtracting gestational age in days from the childbirth date.

defined as any antibiotics dispensed to the mother within 280 days before conception.* Post-pregnancy exposure was defined as any antibiotics dispensed to the mother at any time from the birth date of cohort child until 280 days after delivery.

Choosing these two-time periods for analysis was based on two assumptions: First, as already noted in Chapter 2, maternal antibiotic use out of pregnancy period may contribute to microbiome dysbiosis[221] and thus may have a role in the development of mood and anxiety disorders. Second, the researcher was interested in exploring whether potential associations were specific to pregnancy or not. The investigator postulated that if the relationship between maternal antibiotic use and the development of mood and anxiety disorders was causal, the observed association should be strongest for prenatal antibiotic use. But if the maternal antibiotic use was only a surrogate measure of her lifestyle or genetic susceptibility to infections, then the researcher should expect a similar association for maternal antibiotic use out of the pregnancy period. Since there was no evidence in the literature to recommend the appropriate length of time to use for this purpose, the researcher chose a time frame equal to the normal pregnancy length (280 days) for both pre-gestational and postpartum exposures. Stockholm et al. selected a time frame of 80 weeks to analyze the association between maternal use of antibiotics out of pregnancy period and childhood asthma.[349] Their selection was not evidence-based.

Along with modelling some specific characteristics of dyads, a Breslow-Day statistic approach[350] was also used to determine if maternal age at first birth, household income, infant sex and the

*Using the varible (prvddt) in DPIN, if the date of filled prescription was between the date of conception and 280 days before conception, it was assumed that the mother had received that antibiotic during pre-preganncy period.

location of residence were potential effect modifiers. In this method, the homogeneity of the odds ratios is tested at different levels of potential modifiers.[350] In the presence of any significant effect modifier, the interaction term approach would be performed.

All data management, programming, and analyses in this dissertation were performed using SAS® statistical analysis software, version 9.4 (SAS Institute Inc., Cary, NC, 2011).

### 3.8 Ethical considerations

No significant ethical issues were identified in this study as it was based on de-identified secondary data with no direct involvement of human subjects. Before the commencement of this study, the researcher received approval from the University of Manitoba Health Research Ethics Board (HREB) and Health Information Privacy Committee (HIPC) in compliance with the Personal Health Information Act (PHIA). This study was conducted at the MCHP, and a researcher agreement was signed with MCHP. To protect the confidentiality of the data, strict adherence to the policies and protocols for researching at the MCHP was followed. The data used for this research study did not contain any identifiable personal information. MCHP administrators had already scrambled PHIN, so individuals were not identifiable. To ensure that individual identification was not disclosed, the data was presented in aggregate form. The usage of the Repository housed at MCHP was fully in compliance with all privacy legislation in Manitoba. The ethics approval is presented in **Appendix 4**.

# Chapter 4

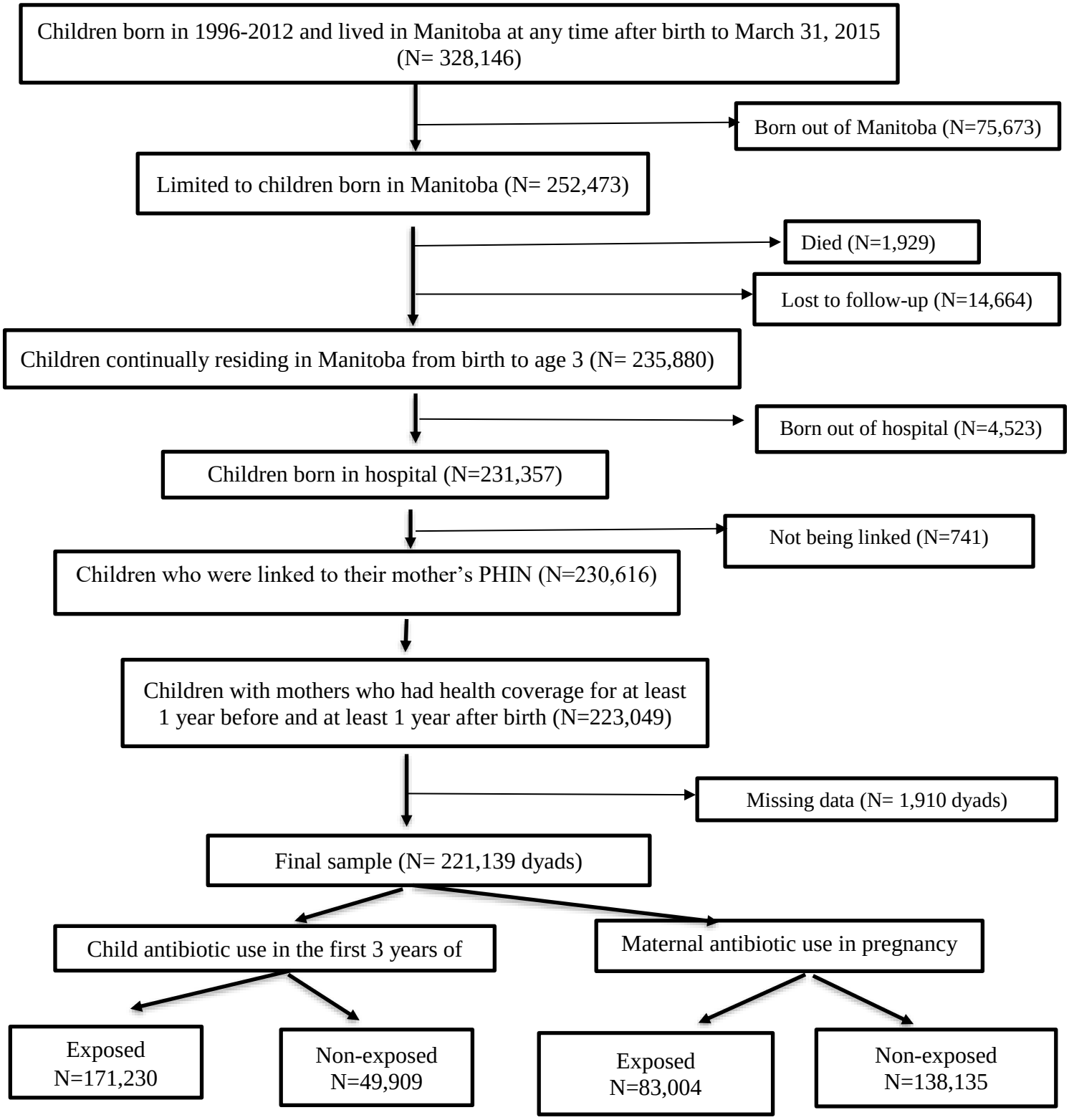
# Results

This chapter includes an overview of descriptive statistics for the study population, prenatal and postnatal cohorts; results from comparing covariates between exposed and non-exposed groups, a summary of how each study variable relates to the outcome variables and results for the final Cox regression model.

## 4.1 Cohort creation

This study was based on an already created provincial birth cohort. A visual description of cohort creation for this study is presented in **Figure 10.** Using scrambled personal identifiers (scrambled PHIN) available in HDAD, mothers and children born alive in Manitoba between January 1, 1996, and December 31, 2012, were linked together. Those children who were lost to follow up due to death or migration before the third birthdate or that could not be linked to their mothers were removed from the initial cohort. This exclusion resulted in 223,049 potential dyads after restricting the study sample to those children who had Manitoba health coverage with a minimum of 3 years from birth, and their mothers had health coverage for at least one year before and at least one year after childbirth. Then the records with missing and invalid data were excluded (n= 1,910) from the study population. The final study sample size was 221,139 linked dyads (from 122,030 different mothers). A prenatal cohort was created for the whole study population assessing the maternal antibiotic prescriptions in pregnancy. A postnatal cohort was also created for the entire population examining the child antibiotic prescriptions in the first three years of life.

**Figure 10- Cohort creation flow-chart**



## 4.2 Descriptive statistics

### 4.2.1 Description of baseline characteristics

The study population consisted of 221,139 children from 122,030 different mothers (221,139 mother-child dyads) with a median follow-up time of 9.4 years [interquartile range (IQR)* 5.3-14.2; Mean 9.8 ± Standard deviation (SD) 5.1] years from the index date. By the end of the follow-up (March 31, 2017), 426 (0.2%) children died, and 9,335 (4.2%) children left the province and could not be followed up.† A summary of baseline characteristics is presented in **Table 3.**

The mean SEFI-2 score was 0.3 (SD 1.1), and most dyads (55.1%) lived in urban settings, and 26.1% of children had a family with a household income in the first income quintile (Q1) area. Most children were male (51.2%) and born via vaginal delivery (80.2%). About 26% of children were born in the summer season. The mean gestational age was 39.1 weeks (SD 1.9), and the mean birth weight was 3,459 grams (SD 570.4). The mean maternal age at delivery was 27.7 years (SD 5.9), and 36.2% of children were the only child of the family with no siblings. About 2.5% of pregnancies resulted in multiple births, and 80% of children were breastfed at hospital discharge. Four percent of children received a diagnosis of mood and anxiety disorders with a mean age of 11 years (SD 3.9).

*From the 25$^{th}$ to 75$^{th}$ percentiles of follow-up time
†These numbers were calculated for children irrespective of being diagnosed or not diagnosed with mood and anxiety disorders.

**Table 3- Descriptive univariate statistics for the whole study population (221,139 mother-infant dyads), Manitoba, Canada, 1996-2012**

| Characteristic | N (%) or Mean ± SD |
|---|---|
| **Gestational age (weeks)** | 39.1 ± 1.9 |
| **Infant birth weight (grams)** | 3,459 ± 570.4 |
| **Maternal age (years)** | 27.7 ± 5.9 |
| **Child age at diagnosis (years)** | 11.0 ± 3.9 |
| **SEFI-2** | 0.3 ± 1.1 |
| **Income quintile** | |
| Q1 (lowest) | 57,673 (26.1) |
| Q2 | 45,733 (20.7) |
| Q3 | 41,700 (18.7) |
| Q4 | 40,523 (18.3) |
| Q5 (highest) | 33,510 (16.2) |
| **Number of children in a household** | |
| 1 | 80,068 (36.2) |
| 2 | 73,780 (33.4) |
| 3 | 35,367 (16.0) |
| 4+ | 31,924 (14.4) |
| **Infant sex** | |
| Male | 113,282 (51.2) |
| Female | 107,857 (48.8) |
| **Residence location** | |
| Rural | 99,340 (44.9) |
| Urban | 121,799 (55.1) |
| **Season of birth** | |
| Spring | 56,306 (25.5) |
| Summer | 58,165 (26.3) |
| Fall | 55,291(25.0) |
| Winter | 51,377 (23.2) |
| **Delivery method** | |
| Cesarean section | 43,794 (19.8) |
| Vaginal | 177,345 (80.2) |
| **Breastfeeding initiation** | 176,814 (80.0) |
| **Multiple births** | 5,615 (2.5) |
| **Child with mood and anxiety disorders** | 8,854 (4.0) |

### 4.2.2 Description of prenatal exposure

Overall 37.5% of mothers received antibiotics during pregnancy, 38% before pregnancy and 40.2% after pregnancy (**Table 4**). Most mothers receiving antibiotics during pregnancy were prescribed a single course (22.2% of all mothers) while fewer received two (8.6%) or more (6.7%) courses during their pregnancy.

Maternal antibiotic use during pregnancy varied slightly by trimester, from 16.9% in the first trimester to 17.9% in the second trimester and 15.9% in the third trimester. β-lactam, penicillin was the most commonly prescribed type of antibiotic (25.3%) with fewer mothers receiving tetracycline, aminoglycosides, quinolones (8.0%); macrolides, lincosamides or streptogramins (7.0%); other β-lactams (6.9%); and sulfonamides and trimethoprim (2.6%). Broad-spectrum antibiotics were more prescribed during pregnancy than narrow-spectrum antibiotics (25.1% vs 20.4%).

**Table 4-Maternal antibiotic use during pregnancy in Manitoba, Canada, 1996-2012 (N=221,139 mother-infant dyads)**

| Characteristic | N | % |
|---|---|---|
| **Any antibiotic use** | | |
| Yes | 83,004 | 37.5 |
| No | 138,135 | 62.5 |
| **Number of antibiotic courses** | | |
| 0 | 138,135 | 62.5 |
| 1 | 49,020 | 22.2 |
| 2 | 19,110 | 8.6 |
| 3 + | 14,874 | 6.7 |
| **Type of antibiotic** | | |
| β-lactam, penicillin | 56,027 | 25.3 |
| Other β-lactams | 15,207 | 6.9 |
| Sulfonamides and trimethoprim | 5,699 | 2.6 |
| Macrolides, lincosamides, streptogramins | 15,394 | 7.0 |
| Tetracycline, aminoglycosides, quinolones, others | 17,700 | 8.0 |
| **Spectrum of antibiotic use** | | |
| Narrow | 45,187 | 20.4 |
| Broad | 55,514 | 25.1 |
| **Timing of maternal antibiotic use** | | |
| 9 months before pregnancy | 83,891 | 38.0 |
| Pregnancy Trimester 1 | 37,331 | 16.9 |
| Pregnancy Trimester 2 | 39,673 | 17.9 |
| Pregnancy Trimester3 | 35,087 | 15.9 |
| 9 months after pregnancy | 88,823 | 40.2 |

### 4.2.3 Comparison of baseline characteristics between prenatally exposed and non-exposed groups

The prenatally exposed children consisted of 83,004 with a median follow-up time of 9.5 years [IQR (5.3- 14.3); Mean 9.8± SD 5.1] years from the index date. The non-exposed group consisted of 138,135 children with a median follow-up time of 9.4 years [IQR (5.3- 14.1); Mean 9.7 ± SD 5.1] years from the index date. The observed differences in the duration of follow-up time were not statistically significant (Std. Diff. =0.0196). Further analyses (**Appendix 5)** also revealed no significant differences in the duration of follow-up time between prenatally exposed and non-exposed children for those who were diagnosed with mood and anxiety disorders or censored due to death or migration (Std. Diff. <0.1).

As presented in **Table 5,** infant sex was similarly distributed between prenatally exposed and non-exposed children. A higher percentage of children with prenatal exposure were living in a rural area compared to non-exposed children (47.6% vs 43.3%). The proportion of cesarean birth was slightly higher among children with prenatal exposure to antibiotics compared to their non-exposed counterparts (20.3% vs 19.5%). The most common season of birth in both exposed (26.6%) and non-exposed children (26.1%) was summer. The proportion of children with no siblings was lower in exposed groups than non-exposed groups (33.5% vs 37.8%). The mean gestational age and birth weight of prenatally exposed and non-exposed children were almost the same. Exposed children were living with families with higher SEFI-2 scores (0.4 ± SD1.1) or lower SES compared to non-exposed children (0.2 ± SD1.2). The mean age at first birth was lower in mothers with antibiotic use in pregnancy (24.9 ± SD 6.0) than mothers with no antibiotic use (26.3 ± SD 5.9). The proportion of children who were breastfed at the time of

discharge was lower in the exposed group (76.7%) compared to the non-exposed group (81.9%). The proportion of multiple births was almost equal in both exposed and non-exposed groups (2.6% vs 2.5%).

The comparison of baseline characteristics between prenatally exposed and non-exposed groups showed a lack of balance in a few variables including maternal age at first childbirth (Std. Diff. = 0.2353), SEFI-2 scores (Std. Diff. = 0.1762), breastfeeding initiation (Std. Diff. = 0.1299) and number of children in the household (Std. Diff. = 0.104). To adjust for the covariate imbalance, all these variables were included in Cox regression modelling. The distribution of additional covariates can be found in **Appendix 6**.

**Table 5- Distribution of covariates between exposed and non-exposed pregnancies (N= 221,139 mother-infant dyads)**

| **Characteristics** | n (%)<br>Mean ±SD<br>**Exposed**<br>(N= 83,004) | n (%)<br>Mean ±SD<br>**Non-exposed**<br>(N= 138,135) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| **Infant sex** | | | 0.4559 | 0.0033 |
| Male | 42,605 (51.3) | 70,677 (51.2) | | |
| Female | 40,399 (48.7) | 67,458 (48.8) | | |
| **Residence location** | | | <0.0001* | 0.0852 |
| Rural | 39,483 (47.6) | 59,857 (43.3) | | |
| Urban | 43,521 (52.4) | 78,278 (56.7) | | |
| **Delivery method** | | | < 0.0001* | 0.0204 |
| Vaginal | 66,144 (79.7) | 111,201 (80.5) | | |
| Cesarean section | 16,860 (20.3) | 26,934 (19.5) | | |
| **Season of birth** | | | 0.0032* | 0.0109 |
| Spring | 21,301 (25.7) | 35,005 (25.4) | | |
| Summer | 22,052 (26.6) | 36,113 (26.1) | | |
| Fall | 20,437 (24.6) | 34,854 (25.2) | | |
| Winter | 19,214 (23.1) | 32,163 (23.3) | | |
| **SEFI2** | 0.4 ± 1.1 | 0.2 ± 1.2 | <0.0001* | 0.1762* |
| **Number of children in household** | | | <0.0001* | 0.104* |
| 1 | 27,829 (33.5) | 52,239 (37.8) | | |
| 2 | 27,368 (33.0) | 46,412 (33.6) | | |
| 3 | 14,204 (17.1) | 21,163 (15.3) | | |
| 4+ | 13,603 (16.4) | 18,321 (13.3) | | |
| **Gestational age (weeks)** | 39.0± 1.9 | 39.1 ± 1.8 | <0.0001* | 0.054 |
| **Birth weight in (grams)** | 3465±576.0 | 3456 ±567.0 | <0.0001* | 0.0158 |
| **Maternal age at first childbirth (years)** | 24.9 ± 6.0 | 26.3± 5.9 | <0.0001* | 0.2353* |
| **Breastfeeding initiation** | | | < 0.0001* | 0.1299* |
| Yes | 63,675 (76.7) | 113,139 (81.9) | | |
| No | 19,329 (23.3) | 24,996 (18.1) | | |
| **Number of births per pregnancy** | | | 0.0814 | 0.0236 |
| Multiple | 2,170 (2.6) | 3,445 (2.5) | | |
| Single | 80,834 (97.4) | 134,690 (97.5) | | |

*Statistically significant

### 4.2.4 Description of postnatal exposure

Overall, 77.4% of children received antibiotics during the first three years of life (0 to 36 months) varying from 46.7% in the first year (0- 12 months), 58.2% in the second year (> 12-24 months) and 48.4% in the third year (> 25-36 months) of life (**Table 6**). About 48% of children receiving antibiotics were prescribed three or more courses while fewer received a single (16.6%) or two (12.9%) courses during the first three years of life. In the first 3 years of life, β-lactams, penicillin were the most commonly prescribed type of antibiotic (69.9%) with fewer children receiving macrolides, lincosamides or streptogramins (31%); other β-lactams (28.2%); sulfonamides and trimethoprim (14.8%); and tetracycline, aminoglycosides, quinolones (3.7%). During the first three years of life, broad-spectrum antibiotics were more prescribed than narrow-spectrum antibiotics (70.9% vs 39.3%). Regarding antibiotic class and spectrum, similar patterns were observed for each year of life as β-lactams, penicillin and broad-spectrum antibiotics were the most commonly prescribed antibiotics during the first, second, and third years of life.

**Table 6- Child antibiotic use in Manitoba, Canada, 1996-2012 (N= 221,139 mother-infant dyads)**

| **Age of Child in months** | **0-12** | | **>12 -24** | | **>24-36** | | **0-36** | |
|---|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | n | % |
| **Any antibiotic use** | | | | | | | | |
| Yes | 103,174 | 46.7 | 128,756 | 58.2 | 107,060 | 48.4 | 171,230 | 77.4 |
| No | 117,965 | 53.3 | 92,383 | 41.8 | 114,079 | 51.6 | 49,909 | 22.6 |
| **Number of antibiotic courses** | | | | | | | | |
| 0 | 117,965 | 53.3 | 92,383 | 41.8 | 114,079 | 51.6 | 49,909 | 22.6 |
| 1 | 47,233 | 21.4 | 49,265 | 22.3 | 49,264 | 22.3 | 36,687 | 16.6 |
| 2 | 24,521 | 11.1 | 29,811 | 13.5 | 26,202 | 11.6 | 28,590 | 12.9 |
| 3 + | 31,420 | 14.2 | 49,680 | 22.5 | 31,594 | 14.3 | 105,953 | 47.9 |
| **Type of antibiotic used** | | | | | | | | |
| β-lactam, penicillin | 86,008 | 38.9 | 105,856 | 47.9 | 82,608 | 37.4 | 154,491 | 69.9 |
| Other β-lactams | 22,030 | 10.0 | 34,360 | 15.5 | 25,942 | 11.7 | 62,353 | 28.2 |
| Sulfonamides and trimethoprim | 14,033 | 6.4 | 16,329 | 7.4 | 11,109 | 5.0 | 32,772 | 14.8 |
| Macrolides, lincosamides, Streptogramins | 24,450 | 11.1 | 39,174 | 17.7 | 30,461 | 13.8 | 68,477 | 31.0 |
| Tetracycline, aminoglycosides, quinolones, others | 3,708 | 1.7 | 4,028 | 1.8 | 2,095 | 1.0 | 8,224 | 3.7 |
| **Spectrum of antibiotic used** | | | | | | | | |
| Narrow | 3,1378 | 14.2 | 49,844 | 22.5 | 41,656 | 18.8 | 86,779 | 39.3 |
| Broad | 89,493 | 40.5 | 109,774 | 49.6 | 85,515 | 38.7 | 156,817 | 70.9 |

### 4.2.5 Comparison of baseline characteristics between postnatally exposed and non-exposed groups

The postnatally exposed children consisted of 171,230 with a median follow-up time of 10.1 years [IQR (5.7- 14.6); Mean 10.2 ± SD 5.1] years from the index date. The non-exposed group consisted of 49,909 children with a median follow-up time of 7.5 years [IQR (4.1- 12.1); Mean 8.3 ± SD 4.9] years from the index date. The observed differences in the duration of follow-up time were statistically significant (Std. Diff. = 0.3842). Further analysis **(Appendix 7)** revealed no significant difference in the duration of follow-up time between exposed and non-exposed groups for those who were diagnosed with mood and anxiety disorders (Std. Diff. = 0.0286). Postnatally exposed children who died or migrated had significantly longer follow-up time compared to non-exposed children (Std. Diff. > 0.1). The impact of the variation in follow-up time for those who died and migrated will be addressed in the sensitivity analyses section.

As presented in **Table 7**, male sex (52.2%) and living in the urban area (56.8%) were more prevalent in children who received antibiotics during the first three years of life compared to those who did not receive any antibiotics. The proportion of cesarean birth was slightly higher amongst children who were exposed to antibiotics in early childhood compared to their non-exposed counterparts (20.1% vs 18.7%). Summer was the most common season of birth in exposed children (26.3%) while non-exposed children were born more in the fall (26.7%).

The proportion of breastfeeding initiation and multiple births as well as the mean of gestational age, birth weight and maternal age at first childbirth were similar in both exposed and non-exposed groups of children.

Compared to the non-exposed group (Mean 0.2 ± SD 1.1), postnatally exposed children were born in families with lower SES or higher SEFI-2 scores (Mean 0.4 ± SD 1.3). The proportions of children with no siblings were similar in both exposed and non-exposed groups (36.1% vs 36.5%).

As reported in **Table 7**, comparing baseline characteristics between postnatally exposed and non-exposed groups showed a lack of balance in a few variables including residence location (Std. Diff. = 0.1265) and SEFI-2 scores (Std. Diff. = 0.1632). To control for the covariate imbalance, all these variables, as well as calendar years were adjusted in Cox regression modelling. The distribution of additional covariates has been presented in **Appendix 8**.

**Table 7- Distribution of covariates between exposed and non-exposed children during the first three years of life (N= 221,139 mother-infant dyads)**

| **Characteristics** | n (%)<br>Mean ± SD<br>**Exposed**<br>(N= 171,230) | n (%)<br>Mean ± SD<br>**Non-exposed**<br>(N= 49,909) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| **Infant sex** | | | <0.0001* | 0.0706 |
| Male | 89,345 (52.2) | 23,937 (48.0) | | |
| Female | 81,885 (47.8) | 25,972 (52.0) | | |
| **Residence location** | | | <0.0001* | 0.1265* |
| Urban | 97,185 (56.8) | 24,614 (49.3) | | |
| Rural | 74,045 (43.2) | 25,295 (50.7) | | |
| **Mode of delivery** | | | <0.0001* | 0.0362 |
| Vaginal | 136,789 (79.9) | 40,556 (81.3) | | |
| Cesarean section | 34,441 (20.1) | 9,353 (18.7) | | |
| **Season of birth** | | | <0.0001* | 0.0136 |
| Spring | 44,041(25.7) | 12,265 (24.6) | | |
| Summer | 45,065 (26.3) | 13,100 (26.2) | | |
| Fall | 41,974 (24.5) | 13,317 (26.7) | | |
| Winter | 40,150 (23.5) | 11,227 (22.5) | | |
| **SEFI2** | 0.4 ± 1.3 | 0.2 ± 1.1 | <0.0001* | 0.1632* |
| **Number of children in household** | | | <0.0001* | 0.0865 |
| 1 | 61,847 (36.1) | 18,221 (36.5) | | |
| 2 | 58,208 (34.0) | 15,572 (31.2) | | |
| 3 | 27,679 (16.2) | 7,688 (15.4) | | |
| 4+ | 23,496 (13.7) | 8,428 (16.9) | | |
| **Gestational age (weeks)** | 39.1± 1.9 | 39.1± 1.8 | 0.0666 | 0 |
| **Birth weight (grams)** | 3,460 ± 537.0 | 3,455 ± 561.0 | 0.0590 | 0.0092 |
| **Maternal age at first childbirth (years)** | 25.8 ± 6.0 | 25.8 ± 6.1 | 0.4885 | 0 |
| **Breastfeeding initiation** | | | 0.1396 | 0.0078 |
| Yes | 137,025 (80.0) | 39,789 (79.7) | | |
| No | 34,205 (20.0) | 10,120 (19.3) | | |
| **Number of births per pregnancy** | | | <0.0001* | 0.0306 |
| Multiple | 4,162 (2.4) | 1,453(2.9) | | |
| Single | 167,068 (97.6) | 48,456 (97.1) | | |

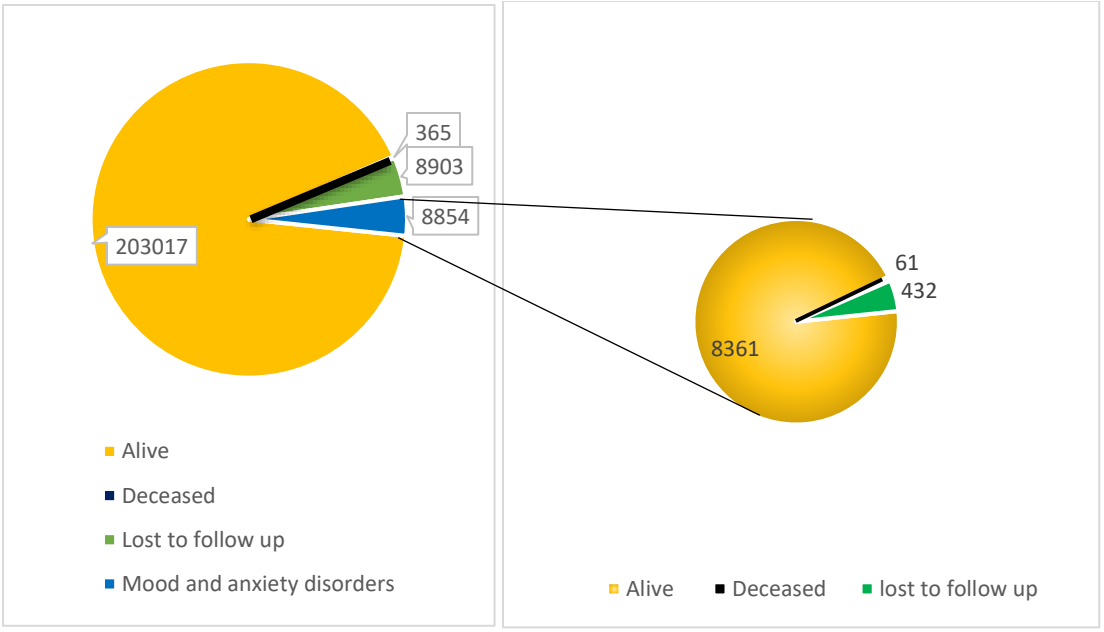
*Statistically significant

### 4.2.6 Mood and anxiety disorders

As depicted in **Figure 11,** of 221,139 children (total study population), 8,854 (4.0%) were diagnosed with mood and anxiety disorders, 365 (0.2%) died, 8,903 (4.0%) were lost to follow up, and 203,017 (91.8%) were still alive at the end of the study period that had not been diagnosed with mood and anxiety disorders yet (Panel A).

Of 8,854 children diagnosed with mood and anxiety disorders, 61 (0.7%) children died, 432 (4.9 %) were lost to follow up, and 8,361 (94.4%) were still alive at the end of the study period. (Panel B).

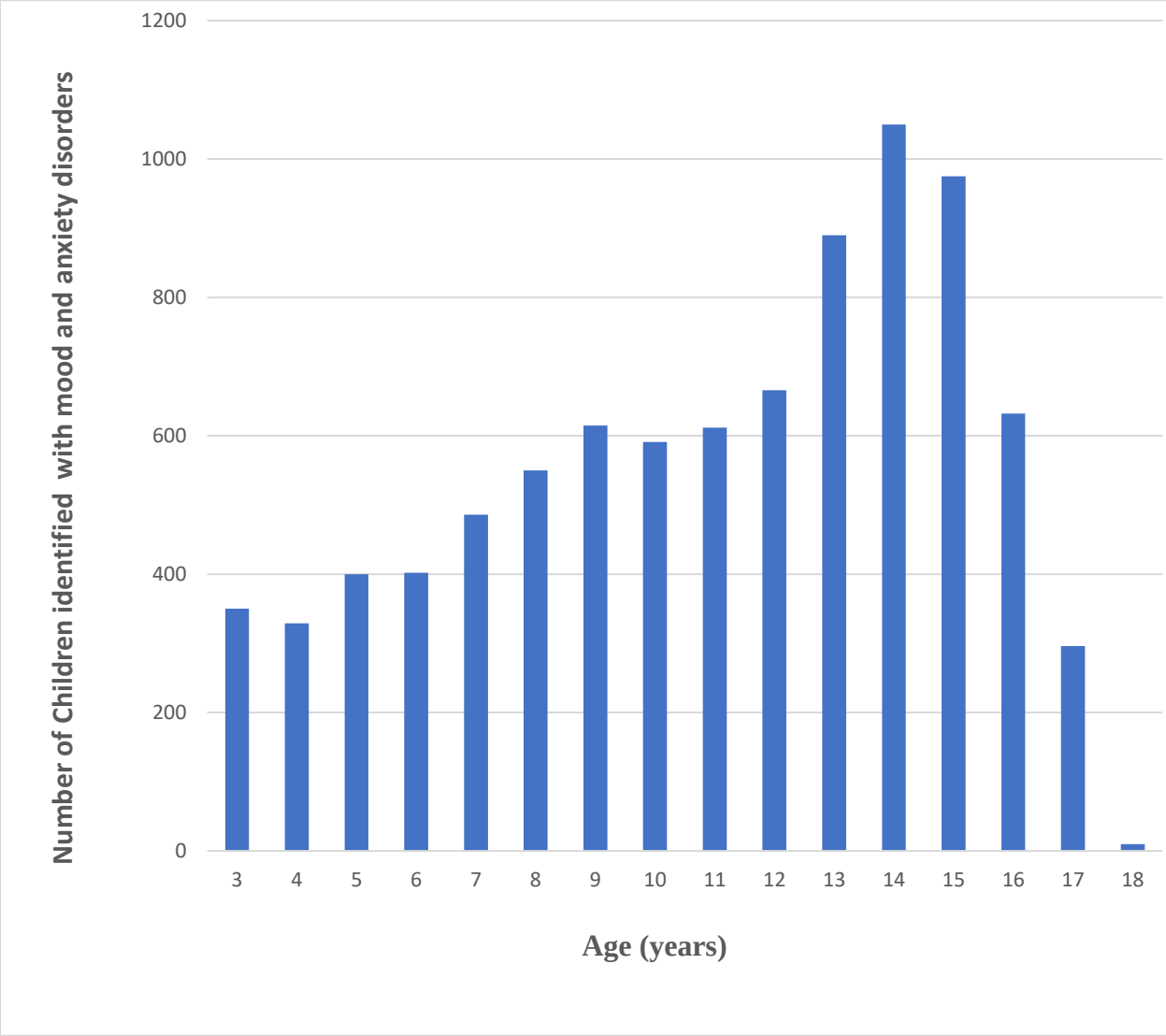
**Figure 11 - Distribution of study population with or without mood and anxiety disorders**




Panel A Panel B

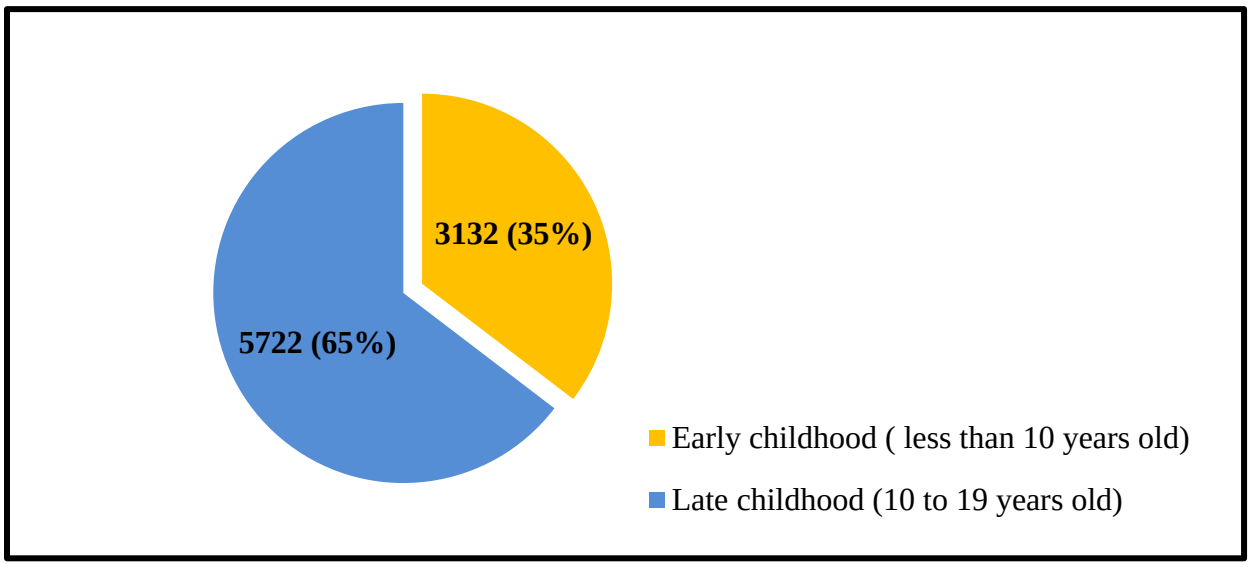
The median age of children when first identified with mood and anxiety disorders was 12.0 [IQR (8-14); Mean 11± SD 3.9] years old. **Figure 12** depicts the age distribution of children when first diagnosed with mood and anxiety disorders. Approximately 4% of mood and anxiety disorders cases were identified at the age of 3 (354 cases) with a maximum incidence of 11.9% at the age of 14 (1,050 cases).

**Figure 12- Age distribution of case children when first identified with mood and anxiety disorders**



As depicted in **Figure 13,** a higher percentage of children were diagnosed with mood and anxiety disorders in late childhood or adolescence (2.6% of total population) than early childhood (1.4% of total population).

**Figure 13- Age distribution of children with mood and anxiety disorders in early and late childhood (N= 8,854)**



As presented in **Table 8**, most children with mood and anxiety disorders were females (57.2%) and lived in an urban setting (65.2%). The proportion of cesarean birth was slightly lower in affected children than non-affected children (18.4% vs 19.9%). Children with mood and anxiety disorders were more likely to be the only child of the family (40.2% vs 36.0%) and have a mother with a history of mood and anxiety disorders (79.7% vs 59.0%) compared to children with no diagnosis of mood and anxiety disorders.

Summer was the most common season of birth in both groups of children (26.3% vs 26,3%). The proportions of breastfeeding initiation (77.1% vs 80.1%) and multiple births (1.9% vs 2.6%) were lower for children with mood and anxiety disorders than other children. The mean gestational age, SEFI-2 scores and birth weight were almost the same in both groups of children. The mothers of children with mood and anxiety disorders were older at the time of their first childbirth (27.7 ± SD 5.9 years) compared to mothers of children without mood and anxiety disorders (26.9 ± SD 6.1 years).

The observed differences between children with and without mood and anxiety disorders were statistically different only for two variables: the maternal history of mood and anxiety disorders (Std. Diff. = 0.1696) and maternal age at first birth (Std. Diff. =0.1307). The distribution of additional covariates can be found in **Appendix 9**.

**Table 8- Distribution of characteristics amongst children with and without mood and anxiety disorders (N= 221,139 dyads)**

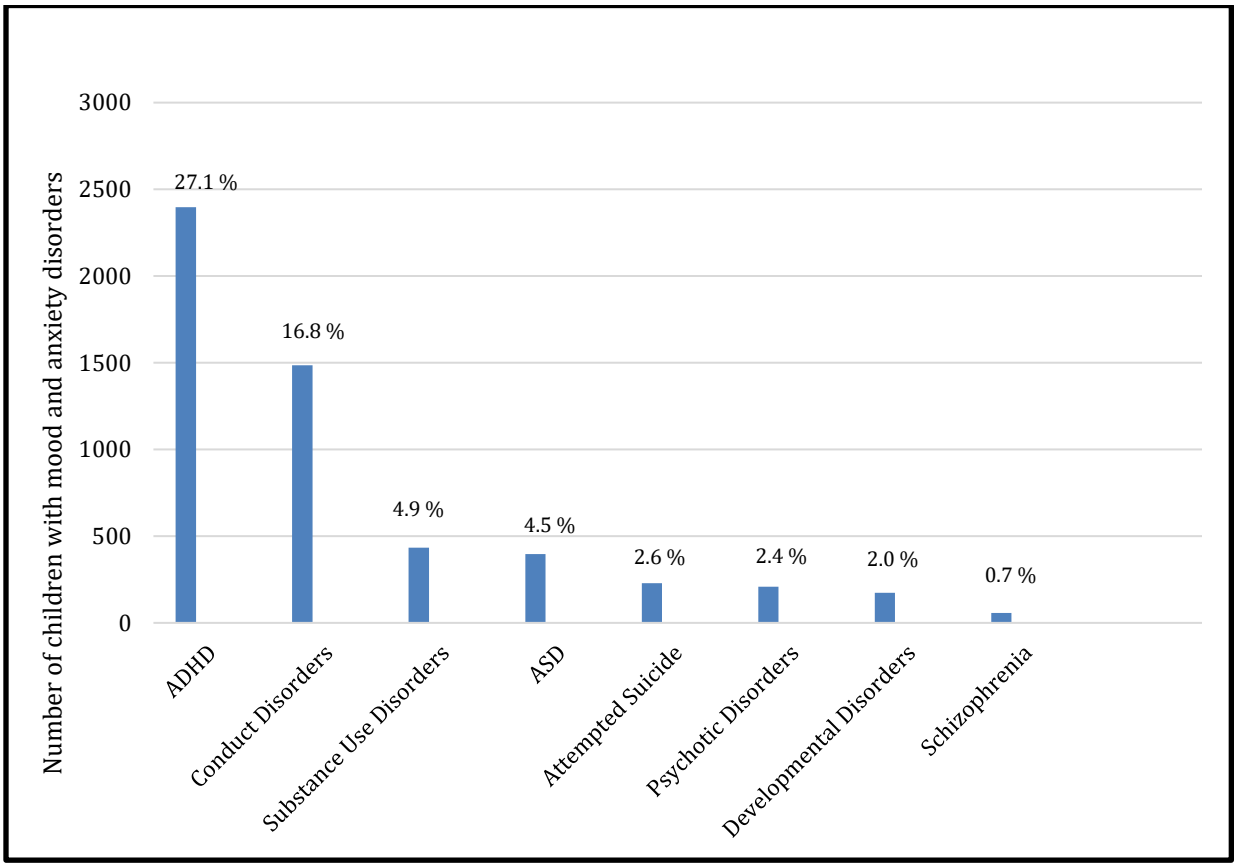
| **Characteristics** | n (%)<br>Mean ± SD<br>**Diagnosed**<br>(N= 8,854) | n (%)<br>Mean ± SD<br>**Not diagnosed**<br>(N=212,285) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| **Infant sex** | | | | |
| Female | 5,062 (57.2) | 102,795 (48.4) | <0.0001* | 0.0687 |
| Male | 3792 (42.8) | 109,490 (51.6) | | |
| **Residence location** | | | | |
| Urban | 5,772 (65.2) | 116,027 (54.7) | <0.0001* | 0.0835 |
| Rural | 3,082 (34.8) | 96,258 (45.3) | | |
| **SEFI2** | 0.27 ± 1.1 | 0.21 ± 1.1 | <0.0001* | 0.0535 |
| **Delivery method** | | | 0.0006* | 0.0184 |
| Cesarean section | 1,627 (18.4) | 42,167 (19.9) | | |
| Vaginal | 7,227 (81.7) | 170,118 (80.1) | | |
| **Season of Birth** | | | 0.1010 | 0.0359 |
| Spring | 2,303 (26.0) | 54,003 (25.4) | | |
| Summer | 2,324 (26.3) | 55,841 (26.3) | | |
| Fall | 2,122 (24.0) | 53,169 (25.1) | | |
| Winter | 2,105 (23.8) | 49,272 (23.2) | | |
| **Number of births per pregnancy** | | | 0.0002* | 0.0498 |
| Multiple | 171 (1.9) | 5,444 (2.6) | | |
| Single | 8,683 (98.1) | 206,841 (97.4) | | |
| **Gestational age (weeks)** | 39.1 (1.9) | 39.1 (1.9) | 1.000 | 0 |
| **Breastfeeding initiation** | | | <0.0001* | 0.0366 |
| Yes | 6,825 (77.1) | 169,989 (80.1) | | |
| No | 2,029 (22.9) | 42,296 (19.9) | | |
| **Birth weight (grams)** | 3,460 ± 570.0 | 3,443 ± 580.0 | 0.0128* | 0.0297 |
| **Number of children in the household** | | | <0.0001* | 0.0911 |
| 1 | 3,556 (40.2) | 76,512 (36.0) | | |
| 2 | 2,950 (33.3) | 70,830 (33.4) | | |
| 3 | 1,243 (14.0) | 34,124 (16.1) | | |
| 4+ | 1,105 (12.5) | 30,819 (14.5) | | |
| **Maternal age at first childbirth (years)** | 27.7 ± 5.9 | 26.9 ± 6.1 | <0.0001* | 0.1307* |
| **Maternal history of mood and anxiety disorders[$]** | | | <0.0001* | 0.1696* |
| Yes | 7,057 (79.7) | 125,197 (59.0) | | |
| No | 1,797 (20.3) | 87,088 (41.0) | | |

*Statistically Significant

[$] Within five years preceding the index date

Children diagnosed with mood and anxiety disorders also received a diagnosis with other mental illnesses. As depicted in **Figure 14**, 27.1% of children with mood and anxiety disorders were diagnosed with ADHD. Conduct disorders were the second common disease (16.8%) followed by substance use disorders (4.9%) and ASD (4.5%). About 2.6 % of children with mood and anxiety disorders attempted suicide. The prevalence of psychotic and developmental disorders in depressed and anxious children was 2.4% and 2.0% respectively. Less than 1% of affected children were diagnosed with schizophrenia.

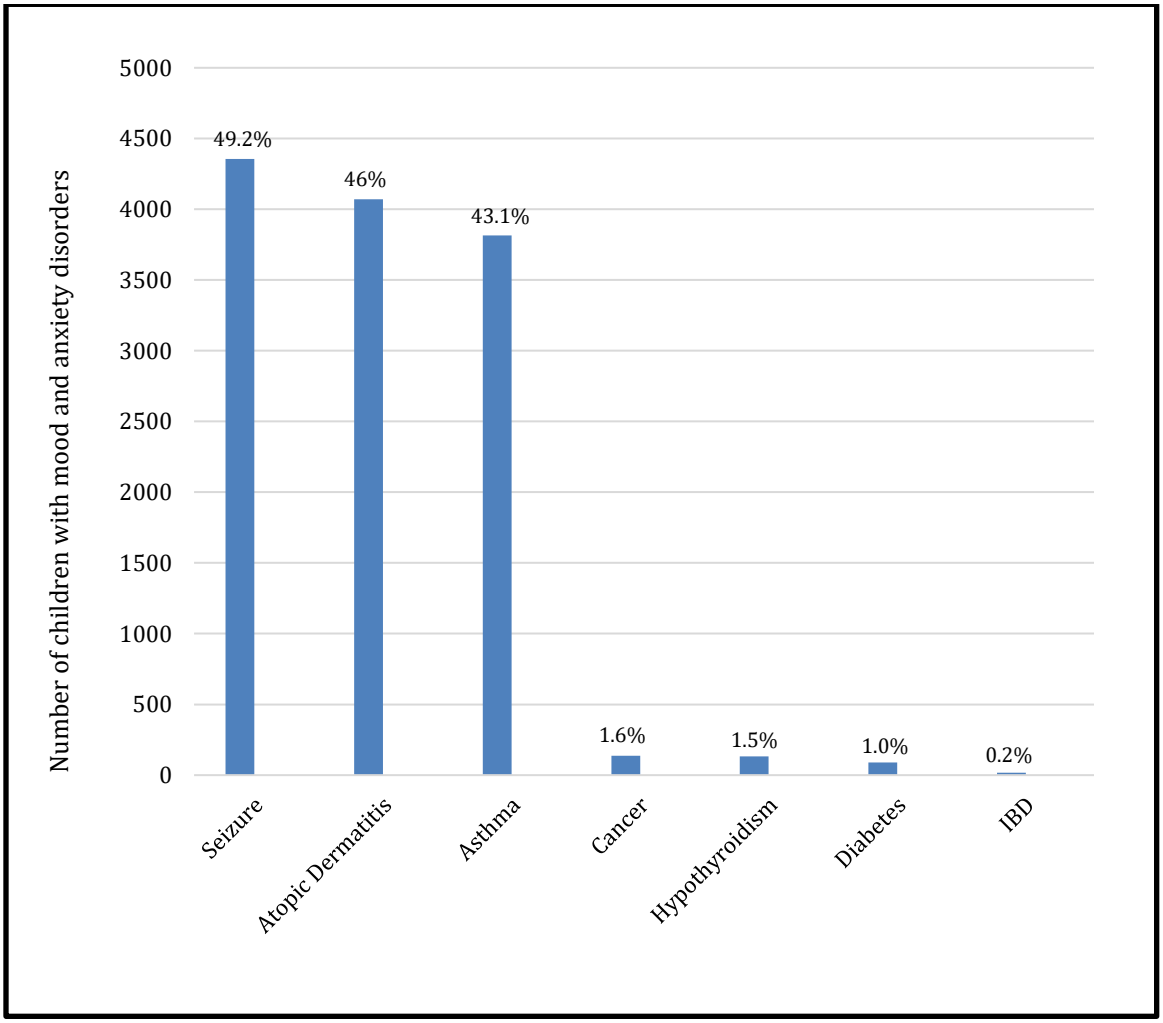
**Figure 14- Distribution of mental illnesses among children with mood and anxiety disorders (N=8854)**




ADHD: Attention Deficit Hyperactivity Disorder
ASD: Autism Spectrum Disorders

As depicted in **Figure 15**, seizure disorder was the most common chronic disease among children with mood and anxiety disorders (49.2%). Atopic dermatitis and asthma were the second and third most common chronic diseases, 46% and 43.1% respectively. The prevalence of cancer, hypothyroidism and diabetes in depressed and anxious children was almost the same, 1.6%, 1.5 % and 1%, respectively. Less than 1% of affected children were diagnosed with IBD.

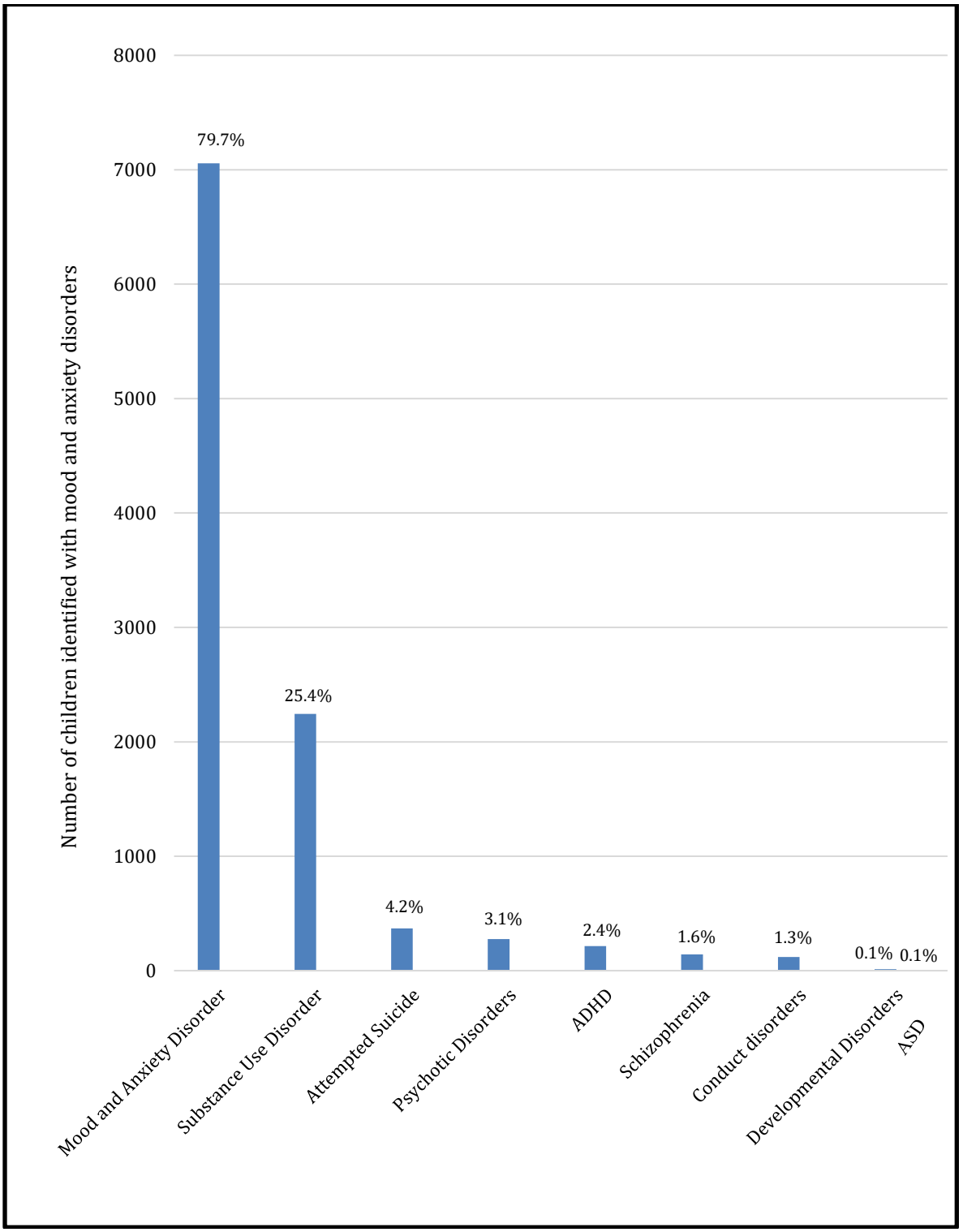
**Figure 15- Distribution of chronic diseases among children with mood and anxiety disorders (N=8,854)**




IBD: Inflammatory Bowel Disease

As already noted, a high percentage of children with mood and anxiety disorders were born to mothers with a history of mood and anxiety disorders (79.7%). About 4.2% of affected children had a mother who attempted suicide. Maternal history of substance use disorder (25.4%), psychotic disorders (3.1%), ADHD (2.4%), schizophrenia (1.6%), conduct disorders (1.3%), ASD (0.1%) and developmental disorders (0.1%) were also recorded among children with mood and anxiety disorders (**Figure 16**).

**Figure 16 - Distribution of maternal history of mental illnesses among children with mood and anxiety disorders (N=8,854)**




ADHD: Attention deficient hyperactivity disorder; ASD: Autism spectrum disorder

### 4.3 Inferential statistics

#### 4.3.1 Prenatal exposure and mood and anxiety disorders

Overall, there were 203,017 (91.8%) children who reached the end of follow-up with no development of mood and anxiety disorders, and 8,903 (4.0%) others were censored due to lost to follow up (migration). There were also 365 deaths (0.2%). There were 3,990 children exposed to antibiotics prenatally (4.8%) compared to 4,864 children with no exposure to prenatal antibiotics (3.5%) that had a physician-diagnosed mood and anxiety disorder within the study period (**Table 9**).

**Table 9- Outcome and censoring events for the survival analysis of the study population in prenatal exposure (N= 221,139)**

| **Outcomes** | n (%)<br>**Exposed**<br>(N= 83,004) | n (%)<br>**Non-exposed**<br>(N= 138,135) | n (%)<br>**Total**<br>(N= 221,139) |
|---|---|---|---|
| Developed mood and anxiety disorders | 3,990 (4.8) | 4,864 (3.5) | 8,854 (4.0) |
| Censoring reasons | | | |
| End of follow up | 75,437 (90.9) | 127,580 (92.4) | 203,017 (91.8) |
| Death | 142 (0.2) | 223 (0.2) | 365 (0.2) |
| Lost to follow up | 3,435 (4.1) | 5,468 (3.9) | 8,903 (4.0) |

##### 4.3.1.1 Results of bivariate analyses

Results from Chi-square test showed that mood and anxiety disorders were significantly more common in children who were exposed prenatally (4.8%) to antibiotics compared to their non-exposed (3.5%) counterparts (*p*-value <0.0001).

As presented in **Table 10**, female and male children were equally exposed to maternal antibiotic use during pregnancy (37.5% vs 37.6%), although the incidence of mood and anxiety disorders was higher among females than males (4.7% vs 3.4%).

The frequency of prenatal exposure to antibiotics was higher among rural residences (39.8% vs 35.7%) while the percentage of mood and anxiety disorders were higher among urban residents (4.7% vs 3.1%) compared to their counterparts.

Cesarean birth was positively associated with maternal antibiotic use but inversely associated with child mood and anxiety. Children born via cesarean were more likely to be exposed to maternal antibiotic use during pregnancy (38.5% vs 37.3%), but they were less likely to receive a diagnosis for mood and anxiety disorders (3.7% vs 4.1%).

Lower income was positively associated with both increased maternal antibiotic use and child mood and anxiety disorders. Except for Q5, the proportion of prenatal exposure decreased consistently with rising household income from 42.7% in Q1 (the lowest level) to 34.0% in Q4. Children born to families with high income (Q4) had the lowest incidence of mood and anxiety disorders (3.8%) while the highest percentage of the disorders (4.3%) was observed in families with the lowest level of income (Q1).

A higher number of siblings was associated with maternal antibiotic use but inversely associated with child mood and anxiety disorders. The proportion of prenatal exposure increased consistently with the increasing number of siblings from 34.8% in families with one child, to 42.6% in families with four or more children. Children with no siblings were less likely to be exposed to prenatal antibiotic exposure (34.8%) but were more likely to develop mood and anxiety disorders (4.4%) compared to children with one or more siblings.

Maternal age at first birth was positively associated with maternal antibiotic use but inversely associated with child mood and anxiety disorders. The proportion of prenatal antibiotic exposure decreased with increasing age of mother at first childbirth. But the incidence of mood and anxiety disorders in offspring increased consistently with increasing age of mother at first childbirth. Accordingly, mothers who were less than 20 years at their first childbirth (46.5%) were more likely to receive antibiotics in pregnancy compared to mothers at other age groups but mothers who experienced their first childbirth at older age (≥35) were more likely (4.9%) to have a child with a mood and anxiety disorders compared to mothers at younger age groups.

Breastfeeding initiation was negatively associated with both maternal antibiotic use and child mood and anxiety disorders. Children who were breastfed at hospital discharge were less likely to be both diagnosed with mood and anxiety disorders (3.9% vs 4.6%) and exposed to maternal antibiotics during pregnancy compared to children who were not breastfed (36.0% vs 43.6%).

Singletons were less likely to be exposed prenatally to antibiotics (37.5% vs 38.7%) but were more likely to develop mood and anxiety disorders compared to their counterparts (4.0% vs 3.1%).

Mothers with a history of mood and anxiety disorders were more likely to receive antibiotics during pregnancy (42.7% vs 29.9%), and their children had a higher incidence of mood and anxiety disorders (5.3% vs 2.0%).

As presented in **Table 10,** results from the Cochran-Mantel-Haenszel test showed that all observed differences were statistically significant (*p*-value <0.0001). Accordingly, all these potential confounders were incorporated into multivariable models to determine the independent association of maternal antibiotic use and child mood and anxiety disorders.

The results of the Breslow-Day test also showed no evidence of effect modification by sex (*p*=0.9372), residence location (*p*=0.4918), maternal age at first birth (*p*= 0.3067) and household income (*p*= 0.1062).

**Table 10- Distribution of covariates among children with mood disorders and maternal antibiotic use in pregnancy (N= 221,139 mother-infant dyads)**

| **Characteristic** | | **Total** (N=221139) | n (%) **Prenatal exposure** (N=83,004) | n (%) **Mood and anxiety disorders** (N= 8,854**)** | ***p*-value** |
|---|---|---|---|---|---|
| **Infant sex** | Male | 113,282 | 42,605 (37.6) | 3,792(3.4) | < 0.0001* |
| | Female | 107,857 | 40,399 (37.5) | 5,062 (4.7) | |
| **Residence Location** | Rural | 99,340 | 39,483 (39.8) | 3,082 (3.1) | < 0.0001* |
| | Urban | 121,799 | 43,521 (35.7) | 5,772 (4.7) | |
| **Delivery method** | Cesarean section | 43,794 | 16,860 (38.5) | 1,627 (3.7) | < 0.0001* |
| | Vaginal | 177,345 | 66,144 (37.3) | 7,227 (4.1) | |
| **Income quintile** | Q1 (lowest) | 57,673 | 24,647 (42.7) | 2,449 (4.3) | < 0.0001* |
| | Q2 | 45,733 | 17,520 (38.3) | 1,828 (4.0) | |
| | Q3 | 41,700 | 15,304 (36.7) | 1,728 (4.1) | |
| | Q4 | 40,523 | 13,773 (34.0) | 1,540 (3.8) | |
| | Q5 (highest) | 33,510 | 11,535 (34.4) | 1,309 (3.9) | |
| **Number of children in the household** | 1 | 80,068 | 27,829 (34.8) | 3,556 (4.4) | < 0.0001* |
| | 2 | 73,780 | 27,368 (37.1) | 2,950 (4.0) | |
| | 3 | 35,367 | 14,204 (40.2) | 1,243 (3.5) | |
| | 4+ | 31,924 | 13,603 (42.6) | 1,105 (3.5) | |
| **Maternal age at first childbirth (years)** | <20 | 40,015 | 18,588 (46.5) | 1,416 (3.5) | < 0.0001* |
| | 20-35 | 163,301 | 58,789 (36.0) | 6,574 (4.0) | |
| | >35 | 17,823 | 5,627 (31.6) | 864 (4.9) | |
| **Breastfeeding** | No | 44,325 | 19,329 (43.6) | 2,029 (4.6) | < 0.0001* |
| | Yes | 176,814 | 63,675 (36.0) | 6,825 (3.9) | |
| **Multiple births** | Yes | 5,615 | 2,170 (38.7) | 171 (3.1) | < 0.0001* |
| | No | 215,524 | 80834 (37.5) | 8,683 (4.0) | |
| **Maternal history of mood and anxiety disorders** ♣ | Yes | 132,254 | 56,475 (42.7) | 7,057 (5.3) | < 0.0001* |
| | No | 88,885 | 26,529 (29.9) | 1,797 (2.0) | |

*Statistically significant

♣ Within five years preceding the index date

#### 4.3.1.2 Results of time-to-event analyses

As illustrated in **Table 11**, the estimated crude hazard ratio for developing child mood and anxiety disorders after prenatal exposure was 1.36 with a 95% CI of 1.30-1.42. The association between prenatal exposure and mood and anxiety disorders remained significant but became weaker following adjustment for maternal age at first birth, household income, breastfeeding initiation, number of births per pregnancy, delivery method, infant sex, location of residence, number of children in the household, child medical comorbidity, calendar year, maternal history of mental health disorders and health care utilization (aHR 1.08, 95% CI 1.03-1.13). Adjusting for child antibiotic use in the first three years of life did not change the results (aHR 1.08, 95% CI 1.03-1.13).

The results from additional analyses also showed that the association between prenatal exposure to antibiotics and development of mood and anxiety disorders remained significant and similar after exposure to two courses of antibiotics (aHR 1.10; 95% CI 1.02 to 1.18), but the associated risk doubled after exposure to three or more courses of antibiotics (aHR 1.19; 95% CI 1.10-1.28).

When classified by type, two classes of antibiotics were similarly and significantly associated with child mood and anxiety disorders including β-lactams, penicillin (n=56,027; aHR 1.07, 95% CI 1.02 - 1.12) and macrolides, lincosamides, streptogramins (n=15,394; aHR 1.08, 95% CI 1.01 - 1.16). As already presented in Table 4, β-lactam, penicillin was the most commonly prescribed type of antibiotic (25.3%) with fewer mothers receiving tetracycline, aminoglycosides,

quinolones (8.0%); macrolides, lincosamides or streptogramins (7.0%); other β-lactams (6.9%); and sulfonamides and trimethoprim (2.6%).

In terms of the timing of exposure, the association of child mood and anxiety disorders with maternal antibiotic use was still significant and almost similar during the second trimester of pregnancy (aHR 1.08, 95% CI 1.03 - 1.14) but the risk slightly increased after exposures during the third trimester of pregnancy (aHR 1.13, 95% CI 1.07 -1.19). There was a similar result for maternal antibiotic use during the nine months after pregnancy (aHR 1.10, 95% CI 1.06 - 1.15).

When examining the spectrum of antibiotic exposure, the risk of mood and anxiety disorders did not change after exposure to broad-spectrum antibiotics (aHR 1.07, 95% CI 1.02- 1.12).

**Table 11- Hazard ratios for the diagnosis of mood and anxiety disorders in children after exposure to antibiotics during pregnancy**

| **Maternal antibiotic use** | **Crude HR** | **Adjusted HR⁂** |
|---|---|---|
| | HR (95% CI) | aHR (95% CI) |
| **Any antibiotic use** | | |
| No | 1.00 (ref) | 1.00 (ref) |
| Yes | 1.36 (1.30 -1.42) * | 1.08 (1.03 - 1.13) * |
| **Number of antibiotic courses** | | |
| 0 | 1.00 (ref) | 1.00 (ref) |
| 1 | 1.22 (1.16 - 1.28) * | 1.04 (0.99 - 1.10) |
| 2 | 1.43 (1.33 - 1.53) * | 1.10 (1.02 - 1.18) * |
| 3+ | 1.73 (1.61 - 1.86) * | 1.19 (1.10 - 1.28) * |
| **Type of antibiotics** | | |
| β-lactam, penicillin | 1.27 (1.22 - 1.33) * | 1.07 (1.02 - 1.12) * |
| Other β-lactams | 1.05 (0.96 - 1.14) | 0.99 (0.91 - 1.07) |
| Sulfonamides and trimethoprim | 1.18 (1.06 - 1.32) * | 1.01 (0.90 - 1.13) |
| Macrolides, lincosamides, streptogramins | 1.34 (1.25 - 1.44) * | 1.08 (1.01 - 1.16) * |
| Tetracyclines, aminoglycosides, quinolones, others | 1.18 (1.10 - 1.27) * | 1.01 (0.94 - 1.09) |
| **Timing of maternal antibiotic use** | | |
| 9 months before pregnancy | 1.27 (1.22 - 1.32) * | 0.99 (0.95 - 1.03) |
| Pregnancy Trimester 1 | 1.20 (1.14 - 1.27) * | 1.00 (0.95 - 1.06) |
| Pregnancy Trimester 2 | 1.26 (1.20 - 1.33) * | 1.08 (1.03 - 1.14) * |
| Pregnancy Trimester3 | 1.28 (1.21 - 1.34) * | 1.13 (1.07 - 1.19) * |
| 9 months after pregnancy | 1.42 (1.36 - 1.48) * | 1.10 (1.06 - 1.15) * |
| **Spectrum of antibiotics** | | |
| Narrow | 1.24 (1.18 - 1.30) * | 1.03 (0.98 - 1.09) |
| Broad | 1.28 (1.22 - 1.34) * | 1.07 (1.02 - 1.12) * |

HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval.
*Statistically significant
⁂Adjusted for maternal age at first birth, household income, breastfeeding initiation, delivery method, infant sex, the location of residence, number of births per pregnancy, number of children in the household, child medical comorbidity, calendar year, maternal history of mental health disorders and health care utilization.

### 4.3.2 Postnatal exposure and mood disorders

Overall, there were 203,017 (91.8%) children who reached the end of follow-up with no development of mood and anxiety disorders, and 8,903 (4.0%) others were censored due to lost to follow up. There were 7,799 children exposed to antibiotics postnatally (4.5%) compared to 1,055 children with no exposure (2.1%) that developed mood and anxiety disorders within the study period (**Table 12**).

**Table 12- Outcome and censoring events for the survival analysis of the study population for postnatal exposure (N= 221139)**

| **Outcomes** | n (%)<br>**Exposed**<br>(N=171,230) | n (%)<br>**Non-exposed**<br>(N= 49,909) | n (%)<br>**Total**<br>(N=221,139) |
|---|---|---|---|
| Developed mood and anxiety disorders | 7,799 (4.5) | 1,055 (2.1) | 8,854 (4.0) |
| Censoring reasons | | | |
| End of follow up | 155,804 (91.0) | 47,213 (94.6) | 203,017 (91.8) |
| Death | 277 (0.2) | 88 (0.2) | 365 (0.2) |
| Migration | 7,350 (4.3) | 1,553 (3.1) | 8,903 (4.0) |

#### 4.3.2.1 Results of bivariate analyses

Results of Chi-square tests showed that mood and anxiety disorders were significantly more common in children who were exposed postnatally (4.5%) to antibiotics compared to their non-exposed (2.1%) counterparts (*p*-value <0.0001).

As presented in **Table 13**, male children were more likely (78.9%) to receive antibiotics in the first three years of life than female children (75.9%); however, the incidence of mood and anxiety disorders was higher among females (4.7% vs 3.4%).

Children living in an urban area were more likely to both receive antibiotics in the postnatal period (79.8% vs 74.5%) and be diagnosed with mood anxiety disorders compared to children living in a rural area (4.7% vs 3.1%).

Cesarean birth was positively associated with childhood antibiotic use but inversely associated with child mood and anxiety disorders. Children born via cesarean were more likely to receive antibiotics in the postnatal period (78.6% vs 77.1%) but were less likely to be diagnosed with mood and anxiety disorders compared to those born via vaginal delivery (3.7% vs 4.1%).

Lower income was associated with decreased postnatal antibiotic exposure and increased child mood and anxiety disorders. The lowest proportion of postnatal exposure (76.9%) was observed amongst children born in a family with the lowest level of income (Q1), but these children had the highest incidence of mood and anxiety disorders (4.3%) compared to children belonging to families with a higher level of income (Q2 to Q5). The highest level of postnatal exposure (82.0%) was observed amongst children with the highest level of household income (Q5).

A higher number of siblings was inversely associated with both child antibiotic use and child mood and anxiety disorders. The lowest proportions of postnatal antibiotic exposure (73.6%) and mood and anxiety disorders (3.5%) were observed in children who had 3 or more siblings. Children with no siblings were at highest risk (4.4%) of developing mood and anxiety disorders compared to children who had one or more siblings.

Maternal age at first birth was positively associated with child mood and anxiety disorders. The incidence of mood and anxiety disorders in offspring increased consistently with increasing age of mother at first childbirth. The proportion of exposed children among mothers in different age groups was almost equal.

Breastfeeding initiation was negatively associated with child mood and anxiety disorders. The proportion of postnatal exposure did not differ among children who were breastfed at the time of discharge from hospital (77.5%) and those who were not (77.2%), but breastfed children were less likely to be diagnosed with mood and anxiety disorders (3.9% vs 4.6%).

Singletons were more likely to receive antibiotics in early childhood (77.5% vs 74.1%) and were more likely to develop mood and anxiety disorders compared to their counterparts (4.0% vs 3.1%).

Children born to mothers with a history of mood and anxiety disorders were more likely to receive antibiotics during the first three years of life (82.0% vs 70.7%) and had a higher prevalence of mood and anxiety disorders (5.3% vs 2.0%).

As presented in **Table 13,** results from the Cochran-Mantel-Haenszel statistic showed that all observed differences were statistically significant ($p$-value <0.0001). Accordingly, all potential confounders were incorporated into multivariable models to determine the independent association of child antibiotic use and the risk of developing mood and anxiety disorders.

The results of the Breslow-Day test also showed no evidence of effect modification by sex ($p$=0.8814), residence location ($p$=0.1383), maternal age at first birth ($p$= 0.1839) and household income ($p$= 0.1219).

**Table 13- Distribution of covariates among children with mood disorders and exposure to antibiotics in the first three years of life (N= 221,139 mother-infant dyads)**

| **Characteristic** | | **Total**<br><br>N=221,139 | n (%)<br>**Postnatal exposure**<br>(N= 171,230) | n (%)<br>**Mood and anxiety disorders**<br>(N= 8,854) | ***p*-value** |
|---|---|---|---|---|---|
| **Infant sex** | Male | 113,282 | 89,345 (78.9) | 3,792 (3.4) | <0.0001* |
| | Female | 107,857 | 81,885 (75.9) | 5,062 (4.7) | |
| **Residence Location** | Rural | 99,340 | 74,045 (74.5) | 3,082 (3.1) | <0.0001* |
| | Urban | 121,799 | 97,185 (79.8) | 5,772 (4.7) | |
| **Delivery method** | Cesarean section | 43,794 | 34,441 (78.6) | 1,627 (3.7) | <0.0001* |
| | Vaginal | 177,345 | 13,6789 (77.1) | 7,227 (4.1) | |
| **Income quintile** | Q1 (lowest) | 57,673 | 44,376 (76.9) | 2,449 (4.3) | <0.0001* |
| | Q2 | 45,733 | 35,206 (77.0) | 1,828 (4.0) | |
| | Q3 | 41,700 | 32,584 (78.1) | 1,728 (4.1) | |
| | Q4 | 40,523 | 31,198 (77.0) | 1,540 (3.8) | |
| | Q5 (highest) | 33,510 | 27,487 (82.0) | 1,309 (3.9) | |
| **Number of children in the household** | 1 | 80,068 | 61,847 (77.2) | 3,556 (4.4) | <0.0001* |
| | 2 | 73,780 | 58,208 (78.3) | 2,950 (4.0) | |
| | 3 | 35,367 | 27,679 (78.9) | 1,243 (3.5) | |
| | 4+ | 31,924 | 23,496 (73.6) | 1,105 (3.5) | |
| **Maternal age at first childbirth (years)** | <20 | 40,015 | 30,587 (76.4) | 1,416 (3.5) | <0.0001* |
| | 20-35 | 163,301 | 127,071 (77.8) | 6,574 (4.0) | |
| | >35 | 17,823 | 13,572 (76.2) | 864 (4.9) | |
| **Multiple births** | Yes | 5,615 | 4,162 (74.1) | 171 (3.1) | <0.0001* |
| | No | 215,524 | 167,068 (77.5) | 8,683 (4.0) | |
| **Breastfeeding initiation** | No | 44,325 | 34,205 (77.2) | 2,029 (4.6) | <0.0001* |
| | Yes | 176,814 | 137,025 (77.5) | 6,825 (3.9) | |
| **Maternal history of mood and anxiety disorders ♣** | Yes | 132,254 | 108,387 (82.0) | 7,057 (5.3) | <0.0001* |
| | No | 88,885 | 62,843 (70.7) | 1,797 (2.0) | |

*Statistically significant

♣ Within five years preceding the index date

#### 4.3.2.2 Results of the time-to-event analysis

As illustrated in **Table 14,** the estimated crude hazard ratio for developing child mood and anxiety disorders after postnatal exposure was 1.68 with a 95% CI of 1.58 - 1.79. The risk of developing mood and anxiety disorders decreased and lost its significance following adjustment for maternal age, household income, breastfeeding initiation, number of births per pregnancy, delivery method, infant sex, the location of residence, number of children in the household, child medical comorbidity, calendar year, health care utilization, maternal antibiotic use during pregnancy and history of mental health disorders (aHR 1.00, 95% CI 0.94 -1.07).

The results from additional analyses also showed that the association between postnatal exposure to antibiotics and development of mood and anxiety disorders remained non-significant when examining the risk of mood and anxiety disorders for the different timing of exposures, the number of courses and spectrum of antibiotics.

Analyses according to the subtype of child antibiotic use yielded significantly increased risks for the development of mood and anxiety disorders amongst children who received sulfonamides and trimethoprim (n=32,772; aHR 1.28, 95% CI 1.22- 1.34), and tetracyclines, aminoglycosides, quinolones (n= 8,224; aHR 1.33, 95% CI 1.24 - 1.43). As already presented in table 6, β-lactams, penicillin were the most commonly prescribed type of antibiotic (69.9%) with fewer children receiving macrolides, lincosamides or streptogramins (31%); other β-lactams (28.2%); sulfonamides and trimethoprim (14.8%); and tetracycline, aminoglycosides, quinolones (3.7%).

**Table 14- Hazard ratios for the diagnosis of mood and anxiety disorders in children who received antibiotics during the first three years of life (N= 221,139 mother-infant dyads)**

| **Child antibiotic use** | **Crude HR** | **Adjusted HR⁂** |
|---|---|---|
| | HR (95% CI) | aHR (95% CI) |
| **Any antibiotic use** | | |
| No | 1.00 (ref) | 1.00 (ref) |
| Yes | 1.68 (1.58 - 1.79) * | 1.00 (0.94 -1.07) |
| **Number of antibiotic courses** | | |
| 0 | 1.00 (ref) | 1.00 (ref) |
| 1 | 1.20 (1.10 -1.31) * | 0.96 (0.88- 1.05) |
| 2 | 1.32 (1.21- 1.44) * | 0.94 (0.86 -1.02) |
| 3+ | 1.90 (1.78- 2.03) * | 1.03 (0.96 -1.10) |
| **Type of antibiotic use** | | |
| β-lactam, penicillin | 1.43 (1.35 - 1.51) * | 1.02 (0.96 - 1.08) |
| Other β-lactams | 1.11 (1.06 - 1.16) * | 0.99 (0.94 - 1.04) |
| Sulfonamides & Trimethoprim | 1.51 (1.44 - 1.58) * | 1.28 (1.22- 1.34) * |
| Macrolides, lincosamides, streptogramins | 0.95 (0.91 - 1.00) | 0.84 (0.80 - 0.88) |
| Tetracyclines, aminoglycosides, quinolones, others | 1.60 (1.50 - 1.71) * | 1.33 (1.24 - 1.43) * |
| **Timing of child antibiotic use** | | |
| 0-12m | 1.20 (1.15 - 1.26) * | 1.01 (0.96 -1.06) |
| 12-24m | 1.29 (1.22 -1.35) * | 1.03 (0.98 -1.08) |
| 24-36m | 1.34 (1.28 - 1.40) * | 1.04 (0.99 -1.09) |
| **Spectrum of antibiotics** | | |
| Narrow | 1.06 (1.02 - 1.11) * | 0.87 (0.84 - 0.91) |
| Broad | 1.62 (1.53 - 1.71) * | 1.06 (1.00 - 1.13) |

HR, hazard ratio; aHR, adjusted hazard ratio; CI, confidence interval.
*Statistically significant
⁂Adjusted for maternal age, household income, breastfeeding status, mode of delivery, infant sex, the location of residence, number of births per pregnancy, number of children in the household, child medical comorbidity, calendar year, health care utilization, maternal use of antibiotics and maternal history of mental health disorders.

### 4.3.3 Sensitivity analyses

To address the second assumption of Cox regression hazards modelling as well as the significant difference in follow-up time between postnatally exposed and non-exposed children who died or migrated, two sensitivity analyses were performed. In total, 365 children died, and 8,903 others were lost to follow up during the study period. The first sensitivity analysis was performed for anyone who died before receiving any diagnosis for mood and anxiety disorders. Removal of this subpopulation from the working dataset did not change the estimated HR in both prenatal and postnatal cohorts (**Table 15**).

In the second sensitivity analysis, in addition to those who died (n=365), those who migrated (n=8,903) were also removed from the working dataset (n=9,268 removed). Again, the estimated HR in both prenatal and postnatal cohorts remained unchanged. Therefore, the non-informative nature of censoring events was not violated and the difference in the follow-up time between exposed and non-exposed children who died or migrated did not distort the final estimates in both prenatal and postnatal cohorts.

**Table 15- Sensitivity analysis comparing antibiotic exposure in prenatal and postnatal cohorts for the development of mood and anxiety disorders.**

| Censoring event | Events | Total after removal | Prenatal exposure aHR (95% CI) | Postnatal exposure aHR (95%CI) |
|---|---|---|---|---|
| Died before being diagnosed with mood and anxiety disorders | 365 | 220,774 | 1.08 (1.03- 1.13) | 1.00 (0.94-1.07) |
| Died or lost to follow up before being diagnosed with mood and anxiety disorders | 9,268 | 211,871 | 1.08 (1.03-1.13) | 1.00 (0.94-1.07) |

# Chapter 5

# Discussion

## 5.1 Purpose and overview

This dissertation aimed to understand differences in the risk for development of mood and anxiety disorders following exposure to antibiotics in early life by comparing children who were exposed prenatally/ postnatally to those children who were not. The literature review indicated two studies assessing the role of antibiotics in the development of mental illnesses. One study examined the association between antibiotic use at an unknown period and development of depression, anxiety and psychosis in individuals between 15 to 65 years old.[281] The second study defined an exposure window period for antibiotics (the first years of life) and assessed its relationship with an outcome other than mood and anxiety disorders (autism) in children.[36]

Despite promising results in animal experiments, no human study to date focused on the association between exposure to antibiotics in early life and the risk of subsequent development of mood and anxiety disorders. To address the existing gap in the literature, this dissertation took a novel approach to examine this potential association by comparing children who were exposed to antibiotics in early life (prenatal/ postnatal) with those who were not, assessing different aspects of exposure in analysis and adjusting for a wide range of potential confounders in the study population.

In the next sections, the main objectives of this dissertation will be again outlined, followed by a summary of the results. Then the results from this dissertation will be interpreted using the two previously mentioned theoretical frameworks and will be compared to the findings of two

previous studies on humans. Following this, the limitations and strengths of this work will be presented, with future directions for research and policy making in this field described last.

### 5.2 Current objectives

There were four objectives addressed throughout this dissertation, two focused on the prenatal period, and two focused on the postnatal period. The first objective was to determine whether maternal antibiotic use during pregnancy (prenatal exposure) influences the risk for subsequent development of mood and anxiety disorders in their offspring. The second objective was to determine whether there are differences in the risk of mood and anxiety disorders associated with different classes, spectrums, number of courses and timing of antibiotic exposure in the prenatal period.

The third objective was to determine whether child antibiotic use during the first three years of life (postnatal exposure) influences the risk for subsequent development of mood and anxiety disorders later. The final objective was to explore whether there are differences in the risk of mood and anxiety disorders associated with different classes, spectrums, number of courses and timing of antibiotic exposure in the postnatal period. All four objectives were addressed using time-to-event analyses for the selected covariates.

### 5.3 Summary of results

The findings of this dissertation supported the hypothesis of a higher risk for the development of mood and anxiety disorders after prenatal exposure to antibiotics in comparison to children with no exposure. After adjusting for potential confounders, the final Cox regression model showed a

statistically significant difference in the development of mood and anxiety disorders between exposed and non-exposed children (aHR 1.08, 95% CI 1.03-1.13). This finding suggests that maternal antibiotic use was associated with 8% increased risk of mood and anxiety disorders in the offspring after controlling for child antibiotic use and several established mood and anxiety risk factors. This weak association may indicate that the effect of antibiotic exposure is small, or that the relationship may be due to another confounding. However, examining different aspects of exposure showed that the potential exposure effect was still small and similar when restricted to β-lactam- penicillin (7%); macrolides, lincosamides and streptogramins (8%) but it slightly increased after exposure in the third trimester (13%) and doubled after exposure to three or more courses of antibiotics (19%). The current study also showed that maternal antibiotic use during the nine months after childbirth was associated with an increased risk for mood and anxiety disorders by 10%.

While the prenatal period stood out as a relatively sensitive period, the postnatal period failed to show any significant influence. After adjusting for potential confounders, the final Cox regression model showed no statistically significant difference for the development of mood and anxiety disorders between postnatally exposed and non-exposed children (aHR 1.00, 95% CI 0.94 -1.07). However, further analyses revealed that postnatal exposure to specific classes of antibiotics including sulfonamides and trimethoprim; and tetracyclines, aminoglycosides, quinolones was associated with an increased risk of mood and anxiety disorders by 28% and 33% respectively.

Additionally, the lowest proportion of child antibiotic use was estimated for tetracyclines, aminoglycosides or quinolones (3.7%) followed by sulfonamides and trimethoprim (14.8%). These low frequencies may be the main reason for the non-significant impact of the overall postnatal antibiotic exposure as they contributed less to the overall risk. Notably, these groups of antibiotics failed to demonstrate any significant effect in the prenatal period. Moreover, none of the specified covariates (infant sex, maternal age at first birth, residence location and household income) were found to modify the observed associations in both prenatal and postnatal cohorts.

The statistically significant findings for prenatal exposure to antibiotics overall and postnatal exposure to specific kinds of antibiotics points to either a causal or an indirect relationship that will be discussed in the following section.

### 5.4 Interpretation of results

There was an apparent dose-response for exposures in the prenatal period which may suggest causality. The risk of developing mood and anxiety disorders was 10% after exposure to two courses and increased to 19% after exposure to three or more courses. However, no trend analysis was performed to confirm this potential dose-response relationship. Also, the magnitude of the observed differences between exposed and non-exposed groups was not large enough to suggest any causality (effect size < 2).[293] There were also some unmeasured confounders related to characteristics of dyads, drug regimen and the indication of prescription that were not captured in the administrative databases. Despite that the researcher tried to examine and control for some confounders by adjusting for them in the final modelling, there are some potential explanations that the observed associations are non-causal.

First, the observed associations may have been confounded by a genetic factor which is known to be a significant contributor to mood and anxiety disorders development.[21,97] The main regression models in this dissertation did not account for confounding due to genetic predisposition (hereditary). Except for the maternal history of mental health disorders, the study did not adjust for maternal and paternal full genetic contributions. Moreover, maternal genetic susceptibility to infection (as the target of antibiotic therapy) during pregnancy is a confounder. The similar association observed for maternal antibiotic use in post-pregnancy period indicates that maternal antibiotic use may be a surrogate marker of her lifestyle or genetic susceptibility to infection which could be inherited by offspring[221,349] and increases the vulnerability to develop mood and anxiety disorders. This is a possibility even though the genes conferring risk of maternal infection[120] and child mood and anxiety disorders have yet to be defined.

Genetic variability of children in response to antibiotics was also not captured in this study. It is already well known that individuals are at different level of susceptibility to demonstrate the adverse effect of a medication.[351] Genes can impact the course of a disease which is the primary target of antibiotic therapy. They can also interact with metabolism, absorption, distribution and excretion of antibiotics.[351] Additionally, the gene-drug interaction is influenced by the racial and ethnic background of the study population[351] which was not examined in this dissertation.

Second, the observed associations may have been distorted by the indication of antibiotic prescription (i.e. infection) and its related characteristics including infection severity, subcategories and changes over time. The importance of infections that required three or more courses of antibiotics (as recorded for prenatal exposure) should not be overlooked. As noted in

Chapter 2, infections produce pro-inflammatory cytokines, which cross the blood-brain barrier and affect brain function.[148] Therefore, infections can influence the mental status of the host by inducing pro-inflammatory responses at systemic and CNS levels.[148] These inflammatory responses have been documented in depression and anxiety as well.[150] As already noted in Chapter 3, the developmental brain dysfunction model also illustrates that infections as an insult to the developing brain can have cognitive, and neuro-behavioral manifestations such as mood dysregulation.[286]

A third possibility is the role of bacteria which required antibiotic treatment. There is evidence linking mood disorders with some bacterial infections.[139-141] As noted in Chapter 3, the microbiome-gut-brain cognition theory indicates that infection with enteric pathogens can affect microbiome composition and gut permeability.[149,292] Through these changes, pathogenic bacterial components can alter signaling to the brain and modify its function.[147] A single large study partially addressed this issue by adjusting for previous infectious events and found an increased risk of mental health disorders.[281] The current study used administrative data which lacked the clinical diagnosis and pathogens that were targeted by the prescribed antibiotics.

A fourth hypothesis could be the influence of maternal deficiency in vitamin D, and other nutrients that affect the immune system.[120] Even though there is a lack of literature to support this hypothesis, maternal vitamin D deficiency may increase the risk of both infections in mothers and mood and anxiety disorders in their offspring.

Fifth, since maternal and child health care utilization have been consistently associated in the literature,[352-354] higher maternal antibiotic use may be a marker for an increased propensity of antibiotic use in children.[221] This propensity may be similarly reflected in medical approaches of mothers to their children's illnesses,[221] including mood and anxiety disorders. Accordingly, maternal antibiotic use as a proxy measure for child antibiotic use may influence the diagnosis of mood and anxiety disorders.

While genetic, inflammatory, bacterial and nutritional hypotheses could not be addressed in the administrative database, the current study does not support the fifth explanation since the results remained unchanged following adjustment for child antibiotic use.

With all the above explanations taken together, the findings of this study cannot support a direct causal relationship between early life exposure to antibiotics and subsequent development of mood and anxiety disorders in children and adolescents.

Nevertheless, there are potential biological explanations for the observed associations in this study. As discussed in Chapter 2, antibiotics have the potential to affect brain function by directly inducing mitochondrial dysfunction and GABA inactivation or indirectly through disruption of microbiome composition. [148,185,187,234] The microbiome-gut-brain cognition theory also indicated that antibiotic exposure could alter the composition of the microbiome, change intestinal physiology and subsequently affect the brain function.[149,292] Accordingly, the findings of this study may indicate that exposure to antibiotics in early life had influenced the function of both the developing brain and microbiome establishment. The rapid growth of the brain during

the fetal period, especially in the third trimester,[355] could be one reason why prenatal exposure to antibiotics was significantly associated with mood and anxiety disorder in this study while the postnatal exposure was not.

### 5.5 Comparison with previous studies

There were two previously identified human studies in the literature. One was a case-control study conducted by Lurie et al. (2015) from the United Kingdom.[281] The authors assessed antibiotic exposure and development of depression, anxiety and psychosis in a large population-based study comprising of individuals from 15 to 65 years of age. The authors assessed antibiotic exposure starting one year before the index date without reporting the minim age of recorded exposures. Their study showed an increased risk of depression after exposure to all antibiotic groups with a minimum of an odds ratio (OR) reported for tetracyclines (OR=1.21; 95% CI 1.14 to 1.28). These researchers also found a higher risk of anxiety after exposure to all antibiotic groups with a minimum OR found for penicillin (OR=1.17; 95% CI 1.01 to1.36). Recurrent exposure to penicillin (> 5 courses) increased the risk of depression by 56% and the risk of anxiety by 44% in the study population. Similar to Lurie et al., findings from this dissertation revealed significant rates of risk for depression and anxiety following postnatal exposures to quinolone (43% vs 33 %), and sulfonamides (35% vs 28%) however, the values reported in this dissertation were lower than previously reported for both antibiotic types. Although not confirmed by trend analysis, an apparent dose-response relationship was also observed in both studies. The previous study examined the frequency and type of exposure while this dissertation investigated the timing of exposure as well as frequency, type and spectrum of antibiotics. Additionally, the previous study used conditional logistic modelling adjusted for SES, alcohol

consumption, smoking and previous infections whereas this dissertation used Cox regression modelling to check for potential time-dependent variables and adjusted for many more covariates than the previous study. The researcher in this dissertation also mutually adjusted for maternal and child health care utilization (including hospitalization and physician ambulatory visits) since they can be a surrogate marker of both antibiotic use and mood and anxiety disorders diagnoses. The strength of the previous study was limiting the possibility of confounding by indication (infection) while the administrative database used in this dissertation could not capture that information. The previous study did not report the minimum age of exposure as well as the minimum age of diagnosis. Additionally, their study population included a wide range of age groups from adolescents to the elderly without reporting the increased risk of depression and anxiety for each age group. The use of different study design and other listed limitations of the previous study should be taken into account when comparing the findings of this dissertation to the previous survey.

Focusing on a different type of mental health disorder, Hamad et al. conducted a population-based cohort study in 2018 using administrative health data at MCHP to investigate the association between exposure to antibiotics in the first year of life and development of autism spectrum disorders (ASD).[36] Their study sample included 214,834 children born in Manitoba, Canada, between April 1, 1998, and March 31, 2016. The results indicated that 43.8% of children received antibiotics in the first years of life which was the same prevalence reported in this dissertation (46.7%). They identified 2,965 children with ASD diagnosis. Their results did not support any significant relationship between antibiotic exposure and ASD (aHR 1.03, 95% CI 0.86, 1.23) after adjustment for many confounders including sex, region, health care access,

SES, maternal age at delivery, prenatal antidepressants use, gestational age, medical comorbidities, birth complications, delivery method, multiple births, breastfeeding initiation, birth order, year and season of birth. The Hamad et al.'s study had some limitations. They did not use a validated algorithm for ASD diagnosis and could not assess medication compliance. Even though they examined a different outcome, the underlying mechanisms for ASD and mood and anxiety disorders may be the same due to the co-occurrence of anxiety and ASD.[282] According to the developmental brain dysfunction model, these clinically heterogeneous disorders may share similar pathophysiology and etiological factors. This model also indicated that each of the listed factors including infection as the target of antibiotics therapy has the potential to contribute to the development of a variety of dysfunctional brain disorders.

Moreover, this model illustrated some ASD symptoms as neurobehavioral manifestations of brain dysfunction and supported the notion of the co-occurrence of mood and anxiety disorders with other neurological diseases including ASD. Goodkind et al. conducted a meta-analysis of 193 studies including 15,892 patients diagnosed with six different mental health disorders.[356] They found common biological changes in the gray matter region of the brain of individuals diagnosed with schizophrenia, bipolar disorder, depression, addiction, obsessive-compulsive disorder, and anxiety. It is possible that similar neurobiological changes to be identified for other mental health disorders including ASD. Given that 4.5% of children with mood and anxiety disorders also received an ASD diagnosis in this dissertation, antibiotic-induced dysbiosis may be the common underlying mechanism in both disorders, even though the association for postnatal exposures in the previous study and the current dissertation were not statistically significant.

No other human study was found in the literature that can be compared with the current dissertation as it was the first study to investigate the effect of early life exposure to antibiotics and subsequent development of mood and anxiety disorders in children.

### 5.6 Limitations

Several limitations were identified in this study. The result of this research may not be generalized to individuals who are insured federally (military and federal inmates), or those not eligible for coverage in the Manitoba Health Insurance Registry.

This study used health administrative data which could be subject to recording bias. Variation in training and supervision of physicians can cause differences in coding styles.[357] Incorrect diagnosis by physicians is also possible. Coding errors in billing records and insufficient information on clinical diagnosis may compromise the accuracy of administrative data,[58,358] and eventually produce unreliable statistics.[359]

Another limitation was the lack of information about the indication of antibiotic prescription in DPIN. The reasons behind a prescription are unknown, and they are not straightforward enough to match the prescription with a particular physician visit where it was written.

The investigator could not control for all confounding factors in the analyses. Some potential confounders that are not captured in administrative databases include maternal nutrition (vitamin D deficiency), paternal history of mood and anxiety disorders, family stressors, exclusive

breastfeeding, indirect exposure to antibiotics in breast milk, food or the environment. These factors may be responsible for some of the outcome differences between exposed and non-exposed children. Accordingly, associations derived from this dissertation are not reliable indicators of causal effects.

Confirming the consumption of filled prescriptions was not possible in this study. Because antibiotic prescriptions may not accurately reflect actual use of antibiotics, exposure cases may have been misclassified which can result in bias and shifting the associations toward the null hypothesis. However, this exposure misclassification is non-differential. Moreover, the related database did not capture antibiotics administered in the hospital due to poor quality. Therefore, this study is missing any information related to antibiotic prescriptions which were given to more than 20% of Manitoba women during parturition which may have a potential effect on infant gut colonization.[179] Hamad et al. addressed this issue in a sensitivity analysis by including inpatient prescriptions and found no change in the final risk estimates.[36]

There is also the possibility of non-differential misclassification of the outcome because the algorithm used in this study for identifying mood and anxiety disorders cases was adopted from previous research in MCHP which was not validated independently. Also, the separation of mood and anxiety disorders was not easily defined in administrative data, and the algorithms used in MCHP research had to group these conditions.

Moreover, this study could not determine when mood and anxiety disorders and comorbidity have been developed since the time of getting a disease and being diagnosed with a disease is not necessarily the same. The outcome measures were based only on diagnoses.

Also, health administrative data capture treated diseases using physician claims or inpatient data.[58] Therefore untreated or undiagnosed mood and anxiety disorders were not included in the study analyses. As well, diagnoses offered by psychologists or services provided by nurses, social workers, and counsellors are not captured by Manitoba's administrative databases.

Variations in the antibiotic regimen were another concern. Some characteristics of antibiotic prescriptions such as duration per each course, dose per unit time, distribution of medication over time and their effects on risk estimates were not assessed in this study. Co-medications and antibiotic interactions with other drugs were not examined in this dissertation.

The impact of breastfeeding on infant microbiome and transmission of antibiotics in breastmilk could not be checked because a long follow-up (for about one-year post-birth) was required. When the mother leaves the hospital, the duration of breastfeeding is unknown. Exclusive breastfeeding is also not captured by the administrative data. There is information on breastfeeding initiation in the hospital, but once the baby is discharged, it is not clear if they are exclusively breastfed or not.

Additionally, there was no data on the microbiome and the factors that may alter its composition, (e.g., diet, probiotics, metabolic changes). Accordingly, it was not possible to thoroughly test the

role of microbiome-gut-brain-axis which served as the foundation for the conceptual framework in this dissertation.

### 5.7 Strengths

This study has several important strengths. It was a large unselected population-based study, which captured virtually all children born in Manitoba over a 22-year period. Using administrative data with detailed information enabled the researcher to document mood and anxiety disorders diagnoses and antibiotic exposures objectively. Health administrative databases are "accessible and timely longitudinal data for an entire jurisdiction at relatively little cost" (p. 572)[58] which are free from recall bias with a low rate of loss to follow up.[58,360-362] They also provide the researcher with key details that cannot be well captured by other methods of data collection (e.g., self-report), including the specific antibiotic type, number of courses, spectrum and timing of exposure.

Moreover, the researcher had access to prescription data of all individuals and did not exclude any dyad on any basis other than health coverage which increases the generalizability of current findings.

This study was based on two theoretical frameworks: developmental brain dysfunction theory and microbiome-gut-brain, cognition theory which were discussed in Chapter 3. Even though this study did not aim to test any theory or model, the basic concepts and the linkage among the concepts of those theories, helped the researcher to interpret some of the findings.

Finally, these results confirm previous research identifying some potential risk factors in the development of mood and anxiety disorders including maternal history of mental health disorders, [335] the location of residence,[147] socioeconomic status,[19] maternal age,[116] family size,[332] breastfeeding status,[131,132,222] mode of delivery[179] and child comorbid.[155,157-163]

## 5.8 Future directions

### 5.8.1 Research implications

This study explored a new topic by comparing the risk of developing mood and anxiety disorders between children exposed to antibiotics in early life and non-exposed children. When planning to design future research, one challenging issue identified throughout the MCHP research is the lack of a validated definition for mood and anxiety disorders. The investigator employed a previously used and accepted algorithm in MCHP. However, using a standard algorithm across future studies will benefit any comparison among studies and meta-analyses on this topic.

In addition to creating a standard criterion for mood and anxiety disorders, more detailed information on the antibiotics (as exposure of interest) is essential for future research. Although this dissertation classified antibiotic exposure based on the spectrum, timing, number of courses and type, there are other aspects of exposure such as dose per unit time, duration of regimen and distribution of medication over time that warrants further consideration. The results may change when these aspects are taken into account.

When using administrative healthcare databases, creating a risk score scale for mood and anxiety disorders to present the risk status of children can be beneficial. This scale can be used for

comparing different risk levels between exposed and non-exposed children and can be well standardized after incorporating possible risk factors in administrative data. This scale can be more beneficial for future case-control studies when a disease risk score is used for matching purposes.

Although the investigator controlled for known confounders available in the MCHP databases, future research should also consider all unmeasured confounders in this dissertation. For instance, type and severity of infection as the indication of antibiotic prescription are important unmeasured factors that may significantly change the final risk estimates. When looking at future research designs, a prospective cohort study would be the best as it can provide stronger evidence on the potential relationship between antibiotic exposure and the development of mood and anxiety disorders by measuring this confounder. There are also other confounders that could contribute to the associations described in this study and are worthy of further investigation.

Considering limitations inherent to the design of the current study, prospective longitudinal studies are warranted to examine current findings further and to explore all these factors and their associations with the risk of infection, and mood and anxiety disorders. However, the complex interplay of genetic and environmental factors influencing gut- brain-axis and consequences following any changes in this axis have made any study in this field very challenging.

### 5.8.2 Policy implications

Mental health disorders as one of the most threatening conditions for children worldwide[363] result from the interplay of genetic and environmental factors.[364] To promote the mental health of children, providing a nurturing environment which is free of risk, specifically during early childhood is essential. According to the National Research Council report on mental health in youth, it is critical to take care of children's mental health to prevent the development of psychiatric disorders before they become harmful or untreatable.[365] In general, exploring any risk factor in early childhood will help understand how and when to intervene in supporting the children's mental well-being. It also helps develop public health policies across different sectors to address the unmet needs of children and adolescents' mental health.

Although the current findings did not support a prenatal/postnatal specific causal relationship between antibiotic use and mood and anxiety disorders in children, when it comes to mothers and children's health, even a weak association should warn physicians to avoid unnecessary administration of antibiotics. The new knowledge gleaned from this study also provides a paradigm-shifting message for health care practitioners and parents to be cautious about prescribing and using antibiotics in pregnancy and early childhood. Furthermore, it provides policymakers with critical evidence (even if not strong) for revising best practices and regulations within the health care system about the use of antibiotics in pregnant women and children.

## 5.9 Conclusion

This study found little evidence to support the hypothesis that prenatal exposure to antibiotics overall is associated with higher risk for the development of mood and anxiety disorders compared to children with no exposure. There was also a modestly increased risk of developing mood and anxiety disorders following postnatal exposure to specific types of antibiotics. Due to unmeasured confounders present in the mother and the child as well as limitations inherent in the study, the observed associations are not strong enough to support causality. Given that antibiotics were commonly prescribed for both children and mothers in this study, recognizing even small differences in the prevalence of mood and anxiety disorders in both prenatal and postnatal cohorts, may shed some light on whether there is an underlying association between antibiotic-induced dysbiosis and subsequent development of mood and anxiety disorders. The results of a previous study in humans did not directly focus on assessing early life exposure to antibiotics. However, the results of this dissertation as a novel and unique study added to and expanded upon the previous literature in animal studies that supported a central role for the gut-brain axis in the pathophysiology of psychiatric disorders. Nonetheless, antibiotics will continue to be an essential part of infection control in pregnancy and early childhood when considering the adverse effects of untreated infections in these vulnerable groups. Therefore, ensuring that physicians are prescribing the safest and the minimum dose of antibiotics to prevent possible development of mood and anxiety disorders is a logical deduction from this study's findings. Future studies are warranted to explore and clarify the nature and the underlying mechanisms of any potential association. This research has just scratched the surface of understanding potential associations between early life exposure to antibiotics and development of mood and anxiety disorders in children.

## Appendices

### Appendix 1-ATC codes of antibacterials for systemic use (World Health Organization) [180]

| Main Category | Subgroup | Antibiotic Name | ATC Drug Classification |
|---|---|---|---|
| **Tetracyclines** | | | |
| | | Demeclocycline | J01AA01 |
| | | Doxycycline | J01AA02 |
| | | Chlortetracycline | J01AA03 |
| | | Lymecycline | J01AA04 |
| | | Metacycline | J01AA05 |
| | | Oxytetracycline | J01AA06 |
| | | Tetracycline | J01AA07 |
| | | Minocycline | J01AA08 |
| | | Rolitetracycline | J01AA09 |
| | | Penimepicycline | J01AA10 |
| | | Clomocycline | J01AA11 |
| | | Tigecycline | J01AA12 |
| | | Combinations of tetracyclines | J01AA20 |
| | | Oxytetracycline, combinations | J01AA56 |
| **Amphenicols** | | | |
| | | Chloramphenicol | J01BA01 |
| | | Thiamphenicol | J01BA02 |
| | | Thiamphenicol, combinations | J01BA52 |
| **β-lactam antibacterials, Penicillins** | | | |
| | Penicillins with extended spectrum | | |
| | | Ampicillin | J01CA01 |
| | | Pivampicillin | J01CA02 |
| | | Carbenicillin | J01CA03 |
| | | Amoxicillin | J01CA04 |
| | | Carindacillin | J01CA05 |
| | | Bacampicillin | J01CA06 |
| | | Epicillin | J01CA07 |
| | | Pivmecillinam | J01CA08 |
| | | Azlocillin | J01CA09 |
| | | Mezlocillin | J01CA10 |
| | | Mecillinam | J01CA11 |
| | | Piperacillin | J01CA12 |
| | | Ticarcillin | J01CA13 |
| | | Metampicillin | J01CA14 |
| | | Talampicillin | J01CA15 |

| | | | |
|---|---|---|---|
| | | Sulbenicillin | J01CA16 |
| | | Temocillin | J01CA17 |
| | | Hetacillin | J01CA18 |
| | | Aspoxicillin | J01CA19 |
| | | Combinations | J01CA20 |
| | | Ampicillin. combinations | J01CA51 |
| | Beta-lactamase sensitive penicillins | | |
| | | Benzylpenicillin | J01CE01 |
| | | Phenoxymethylpenicillin | J01CE02 |
| | | Propicillin | J01CE03 |
| | | Azidocillin | J01CE04 |
| | | Pheneticillin | J01CE05 |
| | | Penamecillin | J01CE06 |
| | | Clometocillin | J01CE07 |
| | | Benzathine benzylpenicillin | J01CE08 |
| | | Procaine benzylpenicillin | J01CE09 |
| | | Benzathine phenoxymethylpenicillin | J01CE10 |
| | | Combinations | J01CE30 |
| | Beta-lactamase resistant penicillins | | |
| | | Dicloxacillin | J01CF01 |
| | | Cloxacillin | J01CF02 |
| | | Methicillin | J01CF03 |
| | | Oxacillin | J01CF04 |
| | | Flucloxacillin | J01CF05 |
| | | Nafcillin | J01CF06 |
| | Beta-lactamase inhibitors | | |
| | | Sulbactam | J01CG01 |
| | | Tazobactam | J01CG02 |
| | Combinationas of penicillins, incl. Beta -lactamase inhibitors | | |
| | | Ampicillin and enzyme inhibitor | J01CR01 |
| | | Amoxicillin and enzyme inhibitor | J01CR02 |
| | | Ticarcillin and enzyme inhibitor | J01CR03 |
| | | Sultamicillin | J01CR04 |
| | | Piperacillin and enzyme inhibitor | J01CR05 |
| | | Combinations of penicillin | J01CR50 |
| **Other β-lactam antibacterials** | | | |

| | | | |
|---|---|---|---|
| | First-generation cephalosporins | Cefalexin | J01DB01 |
| | | Cefaloridine | J01DB02 |
| | | Cefalotin | J01DB03 |
| | | Cefazolin | J01DB04 |
| | | Cefadroxil | J01DB05 |
| | | Cefazedone | J01DB06 |
| | | Cefatrizine | J01DB07 |
| | | Cefapirin | J01DB08 |
| | | Cefradine | J01DB09 |
| | | Cefacetrile | J01DB10 |
| | | Cefroxadine | J01DB11 |
| | | Ceftezole | J01DB12 |
| | Second- generation cephalosporins | | |
| | | Cefoxitin | J01DC01 |
| | | Cefuroxime | J01DC02 |
| | | Cefamandole | J01DC03 |
| | | Cefaclor | J01DC04 |
| | | Cefotetan | J01DC05 |
| | | Cefonicid | J01DC06 |
| | | Cefotiam | J01DC07 |
| | | Loracarbef | J01DC08 |
| | | Cefmetazole | J01DC09 |
| | | Cefprozil | J01DC10 |
| | | Ceforanide | J01DC11 |
| | | Cefminox | J01DC12 |
| | | Cefbuperazone | J01DC13 |
| | | Flomoxef | J01DC14 |
| | Third- generation cephalosporins | | |
| | | Cefotaxime | J01DD01 |
| | | Ceftazidime | J01DD02 |
| | | Cefsulodin | J01DD03 |
| | | Ceftriaxone | J01DD04 |
| | | Cefmenoxime | J01DD05 |
| | | Latamoxef | J01DD06 |
| | | Ceftizoxime | J01DD07 |
| | | Cefixime | J01DD08 |
| | | Cefodizime | J01DD09 |
| | | Cefetamet | J01DD10 |
| | | Cefpiramide | J01DD11 |
| | | Cefoperazone | J01DD12 |
| | | Cefpodoxime | J01DD13 |

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|---|---|---|---|
| | | Ceftibuten | J01DD14 |
| | | Cefdinir | J01DD15 |
| | | Cefditoren | J01DD16 |
| | | Cefcapene | J01DD17 |
| | | Cefotaxime, combinations | J01DD51 |
| | | Ceftazidime, combinations | J01DD52 |
| | | Ceftriaxone. combinations | J01DD54 |
| | | Cefoperazone, combinations | J01DD62 |
| | Fourth- generation cephalosporins | | |
| | | Cefepime | J01DE01 |
| | | Cefpirome | J01DE02 |
| | | Cefozopran | J01DE03 |
| | Monobactams | | |
| | | Aztreonam | J01DF01 |
| | | Carumonam | J01DF02 |
| | Carbapenems | | |
| | | Meropenem | J01DH02 |
| | | Ertapenem | J01DH03 |
| | | Doripenem | J01DH04 |
| | | Biapenem | J01DH05 |
| | | Imipenem and enzyme inhibitor | J01DH51 |
| | | Panipenem and betamipron | J01DH55 |
| | Other cephalosporins and penems | | |
| | | Ceftobiprole medocaril | J01DI01 |
| | | Ceftaroline fosamil | J01DI02 |
| | | Faropenem | J01DI03 |
| | | Cefttolozane and enzyme inhibitor | J01DI54 |
| **Sulfonamides and trimethoprim** | | | |
| | Trimethoprim and derivatives | Trimethoprim | J01EA01 |
| | | Brodimoprim | J01EA02 |
| | | Iclaprim | J01EA03 |
| | Short-acting sulfonamides | | |
| | | Sulfadimidine | J01EB01 |
| | | Sulfamethizole | J01EB02 |
| | | Sulfadimidine | J01EB03 |
| | | Sulfapyridine | J01EB04 |
| | | Sulfafurazole | J01EB05 |
| | | Sulfanilamide | J01EB06 |

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|---|---|---|---|
| | | Sulfathiazole | J01EB07 |
| | | Sulfathiourea | J01EB08 |
| | | Combinations | J01EB20 |
| | Intermediate-acting sulfonamides | | |
| | | Sulfamethoxazole | J01EC01 |
| | | Sulfadiazine | J01EC02 |
| | | Sulfamoxole | J01EC03 |
| | | Combinations | J01EC20 |
| | Long-acting sulfonamides | | |
| | | Sulfadimethoxine | J01ED01 |
| | | Sulfalene | J01ED02 |
| | | Sulfametomidine | J01ED03 |
| | | Sulfametoxydiazine | J01ED04 |
| | | Sulfamethoxypyridazine | J01ED05 |
| | | Sulfaperin | J01ED06 |
| | | Sulfamerazine | J01ED07 |
| | | Sulfaphenazole | J01ED08 |
| | | Sulfamazone | J01ED09 |
| | | Combinations | J01ED20 |
| | Combinations of sulfonamides and trimethoprim, derivatives | | |
| | | Sulfamethoxazole and trimethoprim | J01EE01 |
| | | Sulfadiazine and trimethoprim | J01EE02 |
| | | Sulfametrole and trimethoprim | J01EE03 |
| | | Sulfamoxole and trimethoprim | J01EE04 |
| | | Sulfadimidine and trimethoprim | J01EE05 |
| | | Sulfadiazine and trimethoprim | J01EE06 |
| | | Sulfamerazine and trimethoprim | J01EE07 |
| **Macrolides, lincosamides and streptogramins** | | | |
| | Macrolides | | |
| | | Erythromycin | J01FA01 |
| | | Spiramycin | J01FA02 |
| | | Midecamycin | J01FA03 |
| | | Oleandomycin | J01FA05 |
| | | Roxithromycin | J01FA06 |
| | | Josamycin | J01FA07 |
| | | Troleandomycin | J01FA08 |

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|---|---|---|---|
| | | Clarithromycin | J01FA09 |
| | | Azithromycin | J01FA10 |
| | | Miocamycin | J01FA11 |
| | | Rokitomycin | J01FA12 |
| | | Dirithromycin | J01FA13 |
| | | Flurithromycin | J01FA14 |
| | | Telithromycin | J01FA15 |
| | | Solithromycin | J01FA16 |
| | Lincosamides | | |
| | | Clindamycin | J01FF01 |
| | | Lincomycin | J01FF02 |
| | Streptogramins | | |
| | | Pristinamycin | J01FG01 |
| | | Quinupristin/ dalfopristin | J01FG02 |
| **Aminoglycoside antibacterials** | | | |
| | Streptomycin | | |
| | | Streptomycin | J01GA01 |
| | | Streptoduocin | J01GA02 |
| | Other aminoglycosides | | |
| | | Tobramycin | J01GB01 |
| | | Gentamicin | J01GB03 |
| | | Kanamycin | J01GB04 |
| | | Neomycin | J01GB05 |
| | | Amikacin | J01GB06 |
| | | Netilmicin | J01GB07 |
| | | Sisomicin | J01GB08 |
| | | Dibekacin | J01GB09 |
| | | Ribostamycin | J01GB10 |
| | | Isepamicin | J01GB11 |
| | | Arbekacin | J01GB12 |
| | | Bekanamycin | J01GB13 |
| **Quinolone antibacterials** | | | |
| | Fluoroquinolones | | |
| | | Ofloxacin | J01MA01 |
| | | Ciprofloxacin | J01MA02 |
| | | Pefloxacin | J01MA03 |
| | | Enoxacin | J01MA04 |
| | | Temafloxacin | J01MA05 |
| | | Norfloxacin | J01MA06 |
| | | Lomefloxacin | J01MA07 |
| | | Fleroxacin | J01MA08 |
| | | Sparfloxacin | J01MA9 |

| | | | |
|---|---|---|---|
| | | Rufloxacin | J01MA10 |
| | | Grepafloxacin | J01MA11 |
| | | Levofloxacin | J01MA12 |
| | | Trovafloxacin | J01MA13 |
| | | Moxifloxacin | J01MA14 |
| | | Gemifloxacin | J01MA15 |
| | | Gatifloxacin | J01MA16 |
| | | Prulifloxacin | J01MA17 |
| | | Pazufloxacin | J01MA18 |
| | | Garenoxacin | J01MA19 |
| | | Sitafloxacin | J01MA21 |
| | Other quinolones | | |
| | | Rosoxacin | J01MB01 |
| | | Nalidixic acid | J01MB02 |
| | | Peroxidic acid | J01MB03 |
| | | Pipemidic acid | J01MB04 |
| | | Oxolinic acid | J01MB05 |
| | | Cinoxacin | J01MB06 |
| | | Flumequine | J01MB07 |
| | | Nemonoxacin | J01MB08 |
| **Combinations of antibacterials** | | | |
| | Combinations of antibacterials | | |
| | | Penicillins, combinations with other antibacterials | J01RA01 |
| | | Sulfonamides, combinations with other antibacterials(excl.trimetoprim) | J01RA02 |
| | | Cefuroxime and metronidazole | J01RA03 |
| | | Spiramycin and metronidazole | J01RA04 |
| | | Levofloxacin and ornidazole | J01RA05 |
| | | Cefeprime and amikacin | J01RA06 |
| | | Azithromycin, fluconazole, and secnidazole | J01RA07 |
| | | Tetracycline and oleandomycin | J01RA08 |
| | | Ofloxacin and ornidazole | J01RA09 |
| | | Ciprofloxacin and metronidazole | J01RA10 |
| | | Ciprofloxacin and tinidazole | J01RA11 |
| | | Ciprofloxacin and ornidazole | J01RA12 |
| | | Norfloxacin and tinidazole | J01RA13 |
| **Other antibacterials** | | | |

| | | | |
|---|---|---|---|
| | Glycopeptide antibacterials | | |
| | | Vancomycin | J01XA01 |
| | | Teicoplanin | J01XA02 |
| | | Telavancin | J01XA03 |
| | | Dalbavancin | J01XA04 |
| | | Oritavancin | J01XA05 |
| | Polymyxins | | |
| | | Colistin | J01B01 |
| | | Polymyxin B | J01XB02 |
| | Steroid antibacterials | | |
| | | Fusidic acid | J01XC01 |
| | Imidazole derivatives | | |
| | | Metronidazole | J01XD01 |
| | | Tinidazole | J01XD02 |
| | | ornidazole | J01XD03 |
| | Nitrofuran derivatives | | |
| | | Nitrofurantoin | J01XE01 |
| | | Nifurtoinol | J01XE02 |
| | | Furazidin | J01XE03 |
| | | Nitrofurantoin, combinations | J01XE51 |
| | Other antibacterials | | |
| | | Fosfomycin | J01XX01 |
| | | Xibornol | J01XX02 |
| | | Clofoctol | J01XX03 |
| | | Spectinomycin | J01XX04 |
| | | Methenamine | J01XX05 |
| | | Mandelic acid | J01XX06 |
| | | Nitroxoline | J01XX07 |
| | | Linezolid | J01XX08 |
| | | Daptomycin | J01XX09 |
| | | Bacitracin | J01XX10 |
| | | tedizolid | J01XX11 |

**Appendix 2- List of narrow and broad-spectrum antibiotics (adopted from Sarpong et al. 2015)[181]**

| Class of antibiotics | Narrow-spectrum antibiotics | Broad-spectrum antibiotics |
|---|---|---|
| Tetracyclines | demeclocycline, doxycycline, minocycline, tetracycline | |
| Sulfonamides/ trimethoprim | sulfadiazine, sulfamethoxazole, sulfasalazine, sulfisoxazole, sulfamethoxazole-trimethoprim, erythromycin-sulfisoxazole, trimethoprim | |
| Aminoglycosides | gentamicin, neomycin, paromomycin, tobramycin | |
| Polypeptides | bacitracin, polymyxin b sulfate, methenamine, methenamine-sodium acid phosphate | |
| Glycopeptides | vancomycin | |
| Oxazolidinones | linezolid | |
| Lipopeptide | daptomycin | |
| Antimycobacterial | isoniazid, pyrazinamide, colistimethate | |
| Penicillin | penicillin, penicillin v potassium, ticarcillin, amoxicillin, ampicillin, dicloxacillin, penicillin g benzathine, ampicillin-sulbactam | amoxicillin-clavulanate, amoxicillin-clarithromycin-lansoprazole |
| Cephalosporins | cefazolin, cephazolin, cefadroxil, cefalexin, cephalexin, cefradine, cephradine | cefaclor, cefotetan, cefprozil, cefuroxime, cefixime, cefdinir, cefditoren, cefpodoxime, ceftazidime, ceftibuten, ceftriaxone |
| Macrolides | dirithromycin, erythromycin, erythromycin-sulfisoxazole | azithromycin, clarithromycin, telithromycin, amoxicillin-clarithromycin-lansoprazole |
| Lincosamides | | clindamycin |
| Nitrofurans | | furazolidone, nitrofurantoin |
| Carbacephem | | loracarbef, imipenem-cilastatin, meropenem |
| Quinolones | | ciprofloxacin, norfloxacin, ofloxacin, gatifloxacin, levofloxacin, moxifloxacin, gemifloxacin, cinoxacin |
| Rifamycins | | rifampin, rifabutin, rifaximin |
| Others | | fosfomycin, ethambutol, ethionamide, lidocaine-oxytetracycline, metronidazole, tinidazole |

**Appendix 3- Definitions and codes of the physical and mental comorbid indicators**

| Indicator | Definition |
|---|---|
| **Attention deficit hyperactivity disorder (ADHD)** | Following Chartier et al. algorithm[12]<br>1. 1+ hospitalizations with a diagnosis of the hyperkinetic syndrome (ICD-9-CM code 314 or ICD-10 code F90) in one fiscal year, OR,<br>2. 1+ physician claims with a diagnosis of the hyperkinetic syndrome (ICD-9-CM code 314) in one fiscal year, OR,<br>3. 2+ Rx for ADHD drugs in one fiscal year without a diagnosis in the same fiscal year of - conduct disorder (312/F63, F91, F92) - disturbance of emotions (313/F93, F94) - cataplexy/narcolepsy (347/G47.4), OR,<br>4. 1 Rx for ADHD drugs in one fiscal year with a diagnosis of the hyperkinetic syndrome (ICD-9-CM code 314 or ICD-10 code F90) in the previous three years. |
| **Conduct disorder** | Following Chartier et al. algorithm[12]<br>1-One or more hospitalizations with a diagnosis of conduct disorders 1-1-ICD-9: 312<br>1-2-ICD-10: F91 (All F91 codes except F91.3 - oppositional disorder)<br>2-One or more physician visits with a diagnosis of conduct disorders ICD-9: 312 I |
| **Substance use disorders** | Following Chartier et al. algorithm[12]<br>1-one or more hospitalization with a diagnosis for alcohol or drug psychoses, alcohol or drug dependence, or nondependent abuse of drugs: ICD-9-CM codes 291 (alcoholic psychoses), 292 (drug psychoses), 303 (alcohol dependence), 304 (drug dependence), or 305 (nondependent abuse of drugs) or ICD-10-CA codes F10-F19 and F55; OR (Substance Abuse Diagnoses Codes: ICD-9-CM: 291, 292, 303, 304, 305 ICD-10-CA: F10-F19, F55) Z50.2 and Z50.3<br>2-one or more physician visits with a diagnosis for alcohol or drug psychoses, alcohol or drug dependence, or nondependent abuse of drugs: ICD-9-CM codes 291 (alcoholic psychoses), 292 (drug psychoses), 303 (alcohol dependence), 304 (drug dependence), or 305 (nondependent abuse of drugs). |
| **Suicide attempt** | Following Chartier et al. algorithm[12]<br>Suicide Attempts (as defined in the METIS 2010 deliverable) are defined by an inpatient hospitalization with a diagnosis code for suicide and self-inflicted injury or with a diagnosis code for accidental poisoning combined with a psychiatric tariff code from medical claims during a hospital stay or within 30 days of discharge.<br>ICD-9-CM codes: E950-E959<br>1. Accidental Poisoning: E850-E854, E858, E862, E868<br>2. Self-inflicted poisoning: E950-E952<br>3. Self-inflicted injury by hanging, strangulation, and suffocation: E953<br>4. Self-inflicted injury by drowning: E954<br>5. Self-inflicted injury by firearms and explosives: E955<br>6. Self-inflicted injury by smoke, fire, flames, steam, hot vapors and hot objects: E958.1, E958.2<br>7. Self-inflicted injury by cutting and piercing instruments: E956<br>8. Self-inflicted injury by jumping from high places: E957<br>9. Self-inflicted injury by jumping or lying before a moving object: E958.0<br>10. Self-inflicted injury by the crashing of motor vehicle: E958.5<br>11. Self-inflicted injury by other and unspecified means: E958.3, E958.4, E958.6-E958.9<br>12. Poisoning with undetermined intent: |

| | |
|---|---|
| | ICD-10-CA codes:<br>1. Accidental Poisoning: X40-X42, X44, X46, X47<br>2. Self-inflicted poisoning: X60-X69<br>3. Self-inflicted injury by hanging, strangulation, and suffocation: X70<br>4. Self-inflicted injury by drowning: X71<br>5. Self-inflicted injury by firearms and explosives: X72-X75<br>6. Self-inflicted injury by smoke, fire, flames, steam, hot vapors and hot objects: X76, X77<br>7. Self-inflicted injury by cutting and piercing instruments: X78, X79<br>8. Self-inflicted injury by jumping from high places: X80<br>9. Self-inflicted injury by jumping of lying before a moving object: X81<br>10. Self-inflicted injury by crashing motor vehicle: X82<br>11. Self-inflicted injury by other and unspecified means: X83, X84 12. Poisoning with undetermined intent: Y10-Y12, Y16, Y17 NOTE: there were no supporting ICD-9-CM codes included from source: Fransoo et al. (2009) and Martens et al. (2010)<br>13. Late effects of self-inflicted injury: NOTE: there were no supporting ICD-10-CA codes included from source: Fransoo et al. (2009) and Martens et al. (2010) •<br>Ultimately, the overall SUICIDE ATTEMPT DEFINITION will include those PHINs that attempted suicide or were admitted to the hospital for accidental poisoning (supported by a psychiatric tariff code either during the hospital stay or within 30 days post-discharge).<br>If an adolescent has attempted suicide more than once in the study period, only the first attempt will be examined. |
| **Mood and anxiety disorders** | Following Chartier et al. algorithm[12]<br>1. 1+ hospitalizations with diagnosis codes: ICD9 296.1-296.8, 300.0, 300.2, 300.3, 300.4, 300.7, 309 or 311; ICD10 F31-F33, F341, F38.0, F38.1, F40, F41.0, F41.1, F41.2, F41.3, F41.8, F41.9, F42, F43.1, F43.2, F43.8, F53.0, or F93.0 OR,<br>2. 1+ hospitalizations with diagnosis codes: ICD9 300; ICD10 F32, F341, F40, F41, F42, F44, F45.0, F45.1, F45.2, F48, F68.0 or F99 and one or more prescriptions for an antidepressant or mood stabilizer: ATC codes N05AN01, N05BA, N06A, N05BE01 OR,<br>3. 1+ physician visits with a diagnosis code: ICD9 296, 311 OR,<br>4. 1+ physician visits with a diagnosis code: ICD9 300 and one or more prescriptions for an antidepressant or mood stabilizer: ATC codes N05AN01, N05BA, N06A, N05BE01 OR,<br>5. 3+ physician visits with a diagnosis code: ICD9 300, 309. |
| **Psychotic disorders** | Following Chartier et al. algorithm[12]<br>1-One or more hospitalizations with a diagnosis of psychotic disorders:<br>1-1-ICD-9-code - 295 (schizophrenic disorders) or 297 (delusional disorders) or 298 (other nonorganic psychoses)<br>1-2-ICD-10 codes - F11.5, F12.5, F13.5, F14.5, F15.5, F16.5, F17.5, F18.5, F19.5 (psychotic disorders due to opioids, cannabinoids…etc.), F20 (schizophrenia), F22 (delusional disorder), F23 (acute and transient psychotic disorders), F24 (induced delusional disorder), F25 (schizoaffective disorders), F28 (other nonorganic psychotic disorders), F29 (unspecified nonorganic psychosis). |

| | |
|---|---|
| | 2-One or more physician visits with a diagnosis of psychotic disorders: ICD-9-code - 295 (schizophrenic disorders) or 297 (delusional disorders) or 298 (other nonorganic psychoses) |
| **Schizophrenia** | Following Chartier et al. algorithm[12]<br>1-One or more hospitalizations with a diagnosis of schizophrenia (ICD-9-CM code 295; ICD-10-CA codes F20, F21, F23.2, F25)<br>OR<br>2-One or more physician visits with a diagnosis of schizophrenia (ICD-9-CM code 295). |
| **Developmental disorders** | Following Chartier et al. algorithm[12]<br>In the Medical Services data, diagnoses are recorded using three-digit ICD-9-CM diagnosis codes, and therefore the 5-digit, specific codes used in the hospital data are not available from the Medical Services data.<br>The following 3-digit ICD-9-CM codes were used to select cases of Developmental Disability from the Medical Services data:<br>1. 317 = Mild Mental Retardation (MR)<br>2. 318 = Other MR<br>3. 319 = Unspecified MR<br>4. 299 = Autism and other psychoses with origin specific to childhood in the hospital discharge data.<br>The following ICD-10-CA codes were used to select cases of Developmental Disorders (NOTE: In Manitoba, for data beginning on April 1, 2004, up to 25 diagnoses can be coded in an abstract using ICD-10-CA):<br>1. F70.0, F70.1, F70.8, F70.9 = Mild mental retardation;<br>2. F71.0, F71.1, F71.8, F71.9 = Moderate mental retardation;<br>3. F72.0, F72.1, F72.8, F72.9 = Severe mental retardation;<br>4. F73.0, F73.1, F73.8, F73.9 = Profound mental retardation;<br>5. F78.0, F78.1, F78.8, F78.9 = Other mental retardation;<br>6. F79.0, F79.1, F79.8, F79.9 = Unspecified mental retardation;<br>7. F84.0, F84.1, F84.3, F84.4, F84.5, F84.8, F84.9 = Pervasive developmental disorders (PDD);<br>8. Q86.1, Q86.2, Q86.8 = Congenital malformation syndromes due to known exogenous causes, not elsewhere classified;<br>9. Q87.0, Q87.1, Q87.2, Q87.3, Q87.5, Q87.8 = Other specified congenital malformation syndromes affecting multiple systems;<br>10. Q89.8 = Other specified congenital malformations;<br>11. Q90.0, Q90.1, Q90.2, Q90.9 = Down's syndrome;<br>12. Q91.0, Q91.1, 91.2, Q91.3, 91.4, Q91.5, 91.6, Q91.7 = Edward's syndrome and Patau's syndrome;<br>13. Q93.0, Q93.1, Q93.2, Q93.3, Q93.4, Q93.5, Q93.6, Q93.7, Q93.8, Q93.9 = Monosomies and deletions from the autosomes, not elsewhere classified; and<br>14. Q99.2 = Fragile X chromosome (note: P04.3 ‘Fetus and newborn affected by maternal use of alcohol’ presented unreliable coding and therefore was excluded) |
| **Autism spectrum disorders (ASD)** | Following Chartier et al. algorithm[12]<br>An individual was considered to have ASD if he/she had at least one of the following:<br>One or more hospitalizations with any one of the recorded ICD-10-CA diagnostic codes as F84.0-F84.5, F84.8, or F84.9 |

| | |
|---|---|
| | One or more physician claims with the diagnosis code 299 |
| **Child mortality** | All deaths with any reason in the population of children with or without mental health problems during the study period |
| **Co-morbidities** | A diagnosis of any of the following physical health indicators<br>1-Asthma<br>2-Diabetes<br>3-Atopic dermatitis<br>4- Seizure disorder<br>5-IBD<br>6-Hypothyroidism<br>7-Cancer |
| **Asthma** | Following Chartier et al. algorithm[12]<br>1. 1+ Hospitalizations in 2 years (any dx code): ICD-9 (493), or ICD_10-CA (J45) 2. 1+ Physician visits in 2 years (prefix=7): ICD-9 (493) 3. 1+ Prescriptions in 2 years: R03, R06AX17, R05CA10 (with several dins deleted after being reviewed). They are: '01900552', '02394936', '02409720', '02394936', '02418282', '02418401', '00328944', '02359456', '02376938', '02408872' |
| **Diabetes** | Following 't Jong et al. algorithm[366]<br>Any hospitalization for diabetic ketoacidosis or patient in DER-CA database |
| **Atopic dermatitis** | Following Chartier et al. algorithm[12]<br>1-One or more hospitalizations, where one of the 16 or 25 recorded diagnoses was one of:<br>A. ICD9-CM:<br>I. 691: Atopic dermatitis and related conditions,<br>II. 692: Contact dermatitis and another eczema, or<br>III. 693: dermatitis due to substances taken internally (e.g., Food, drugs, other specified, unspecified)<br>B- ICD10-CA:<br>I. L20: Atopic dermatitis,<br>II. L23: Allergic contact dermatitis,<br>III. L25: Unspecified contact dermatitis, or<br>Iv. L27: Dermatitis due to substances taken internally<br>2. At least one physician visit where the diagnosis was either 691, 692 or 693. |
| **Seizure disorder** | Following MCHP algorithm[367]<br>A seizure disorder is defined by a diagnosis or prescription drug for the condition. The diagnosis-based definition is at least one hospitalization or Physician Visit over one year for seizure disorder (ICD-9-CM=345: Epilepsy). The prescription-based definition is at least one prescription over one year for an anti-convulsing medication. |
| **Inflammatory bowel disease (IBD)** | Following Bernstein et al. algorithm[368]<br>*Crohn's Disease (CD)*<br>For an individual registered with Manitoba Health for at least two years, he/she was considered to have CD if he/she had five or more hospitalizations or physician claims with any one of the recorded ICD-9-CM diagnostic codes as 555.<br>For an individual registered with Manitoba Health for less than two years, he/she was considered to have CD if he/she had three or more hospitalizations or physician claims with any one of the recorded ICD-9-CM diagnostic codes as 555. |

| | |
|---|---|
| | *Ulcerative Colitis (UC)*<br>For an individual registered with Manitoba Health for at least two years, he/she was considered to have UC if he/she had five or more hospitalizations or physician claims with any one of the recorded ICD-9-CM diagnostic codes as 556.<br>For an individual registered with Manitoba Health for less than two years, he/she was considered to have UC if he/she had three or more hospitalizations or physician claims with any one of the recorded ICD-9-CM diagnostic codes as 556.<br>If an individual had records that satisfied the criteria for both CD and UC, the nine most recent medical contacts are considered, and the majority diagnosis will be used.<br>*No IBD*<br>An individual was considered to have no IBD if he/she did not have any medical contact related to IBD. |
| **Hypothyroidism** | A child or adolescent is considered to have hypothyroidism if she/ he has received<br>1-at least two prescriptions per year<br>OR<br>2-was diagnosed with any of the following ICD-9-CM with the Elixhauser Comorbidity Index[369]<br>240 Simple and unspecified goiters<br>243 Congenital hypothyroidism<br>244 Acquired hypothyroidism<br>246 Other disorders of thyroid |
| **Cancer** | Using 3-digit ICD-9-CM Codes with the Elixhauser Comorbidity Index for metastatic tumors as 196-199[369] |

## Appendix 4- Ethics approval

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Fax: 204-789-3414

UNIVERSITY OF MANITOBA | Research Ethics - Bannatyne
Office of the Vice-President (Research and International)

# HEALTH RESEARCH ETHICS BOARD (HREB)
## CERTIFICATE OF FINAL APPROVAL FOR NEW STUDIES
### Delegated Review

| PRINCIPAL INVESTIGATOR: | INSTITUTION/DEPARTMENT: | ETHICS #: |
|---|---|---|
| Mahin Delara | U of M and MCHP/Medicine/Applied Health Sciences | HS20896 (H2017:207) |
| **APPROVAL DATE:** June 1, 2017 | **EXPIRY DATE:** June 1, 2018 | |
| **STUDENT PRINCIPAL INVESTIGATOR SUPERVISOR (If applicable):** Dr. Javier Mignone | | |

| PROTOCOL NUMBER: | PROJECT OR PROTOCOL TITLE: |
|---|---|
| N/A | Early life exposure to antibiotics and mental health disorders in childhood and adolescence (an extension of outcomes of HREB 2015:070 without use of additional databases) (Linked to H2015:070) |
| **SPONSORING AGENCIES AND/OR COORDINATING GROUPS:** Children's Hospital Research Institute of Manitoba (CHRIM) | |

| Submission Date of Investigator Documents: | HREB Receipt Date of Documents: |
|---|---|
| May 24, 2017 | May 29, 2017 |

**THE FOLLOWING ARE APPROVED FOR USE:**

| Document Name | Version(if applicable) | Date |
|---|---|---|
| **Protocol:** Protocol | | May 28, 2017 |
| **Consent and Assent Form(s):** | | |
| **Other:** Data Extraction Form (Undated) | | submitted May 24, 2017 |

**CERTIFICATION**
The above named research study/project has been reviewed in a ***delegated manner*** by the University of Manitoba (UM) Health Research Board (HREB) and was found to be acceptable on ethical grounds for research involving human participants. The study/project and documents listed above was granted final approval by the Chair or Acting Chair, UM HREB.

**HREB ATTESTATION**
The University of Manitoba (UM) Research Board (HREB) is organized and operates according to Health Canada/ICH Good Clinical Practices, Tri-Council Policy Statement 2, and the applicable laws and regulations of Manitoba. In respect to clinical trials, the HREB complies with the membership requirements for Research Ethics Boards defined in Division 5

of the Food and Drug Regulations of Canada and carries out its functions in a manner consistent with Good Clinical Practices.

**QUALITY ASSURANCE**
The University of Manitoba Research Quality Management Office may request to review research documentation from this research study/project to demonstrate compliance with this approved protocol and the University of Manitoba Policy on the Ethics of Research Involving Humans.

**CONDITIONS OF APPROVAL:**

1. The study is acceptable on scientific and ethical grounds for the ethics of human use only. ***For logistics of performing the study, approval must be sought from the relevant institution(s).***
2. This research study/project is to be conducted by the local principal investigator listed on this certificate of approval.
3. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to the research study/project, and for ensuring that the authorized research is carried out according to governing law.
4. **This approval is valid until the expiry date noted on this certificate of approval. A Bannatyne Campus Annual Study Status Report** must be submitted to the HREB within 15-30 days of this expiry date.
5. Any changes of the protocol (including recruitment procedures, etc.), informed consent form(s) or documents must be reported to the HREB for consideration in advance of implementation of such changes on the **Bannatyne Campus Research Amendment Form.**
6. Adverse events and unanticipated problems must be reported to the HREB as per Bannatyne Campus Research Boards Standard Operating procedures.
7. The UM HREB must be notified regarding discontinuation or study/project closure on the **Bannatyne Campus Final Study Status Report.**



**Please quote the above Human Ethics Number on all correspondence.**
Inquiries should be directed to the REB Secretary **Telephone:** (204) 789-3255/ **Fax:** (204) 789-3414

**Appendix 5- Bivariate analysis of follow-up time in the prenatal cohort**

| **Population** | *Mean± SD (years)* **Exposed** (N= 83,004) | *Mean± SD (years)* **Non-exposed** (N= 138,135) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| Whole population | 9.8 ± 5.1 | 9.7 ± 5.1 | <.0001* | 0.0196 |
| Diagnosed with mood and anxiety disorders | 15.1 ± 3.6 | 15.1 ± 3.4 | 0.3173 | 0 |
| Censored due to death | 7.7 ± 5.5 | 7.4 ± 5.3 | 0.5785 | 0.0558 |
| Censored due to migration | 4.3 ± 3.7 | 4.0 ± 3.5 | 0.0013* | 0.0838 |

*Statistically significant

**Appendix 6- Distribution of additional covariates between exposed and non-exposed pregnancies (N= 221,139 mother-infant dyads)**

| **Characteristics** | n (%)<br>Mean ±SD<br>**Exposed**<br>(N= 83,004) | n (%)<br>Mean ±SD<br>**Non-exposed**<br>(N= 138,135) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| **Child Medical Comorbidities** | | | | |
| ***Inflammatory bowel disease*** | | | 0.7757 | 0.0014 |
| Yes | 46 (0.1) | 72 (0.1) | | |
| No | 82,985 (99.9) | 138,063 (99.9) | | |
| ***Asthma*** | | | <0.0001* | 0.1166* |
| Yes | 19,102 (23.0) | 2,5347 (18.4) | | |
| No | 63,902 (77.0) | 112,788 (81.6) | | |
| ***Cancer*** | | | 0.1307 | 0.0067 |
| Yes | 415 (0.5) | 758 (0.5) | | |
| No | 82,589 (99.5) | 137,377 (99.5) | | |
| ***Atopic dermatitis*** | | | <0.0001* | 0.0914 |
| Yes | 2,2762 (27.4) | 32,422 (23.5) | | |
| No | 60,242 (72.6) | 105,713 (76.5) | | |
| ***Diabetes*** | | | 0.0563 | 0.0084 |
| Yes | 225 (0.3) | 317 (0.2) | | |
| No | 82,779 (99.7) | 137,818 (99.8) | | |
| ***Seizure disorders*** | | | <0.0001* | 0.0924 |
| Yes | 23,198 (27.9) | 33,053 (23.9) | | |
| No | 59,806 (72.1) | 105,082 (76.1) | | |
| ***Hypothyroidism*** | | | <0.0001* | 0.0202 |
| Yes | 384 (0.5) | 466 (0.3) | | |
| No | 82,620 (99.5) | 137,669 (99.7) | | |
| **Maternal History of Mental Health Disorders** | | | | |
| ***Attention deficit hyperactivity disorder*** | | | <0.0001* | 0.2585* |
| Yes | 1,825 (2.2) | 1,837 (1.3) | | |
| No | 81,179 (97.8) | 136,298 (98.7) | | |
| ***Developmental disorders*** | | | <0.0001* | 0.3868* |
| Yes | 90 (0.1) | 70 (0.1) | | |
| No | 82,914 (99.9) | 138,065 (99.9) | | |
| ***Suicide attempt*** | | | <0.0001* | 0.253* |
| Yes | 2,890 (3.5) | 2,954 (2.1) | | |
| No | 80,114 (96.5) | 135,181 (97.9) | | |
| ***Mood and anxiety disorders*** | | | <0.0001* | 0.2678* |
| Yes | 56,475 (68.0) | 75,779 (54.9) | | |
| No | 26,529 (32.0) | 62,356 (45.1) | | |
| ***Schizophrenia*** | | | <0.0001* | 0.345* |
| Yes | 828 (1.0) | 702 (0.5) | | |

| | | | | |
|---|---|---|---|---|
| No | 82,176 (99.0) | 137,433 (99.5) | | |
| ***Psychotic disorders*** | | | <0.0001* | 0.2477* |
| Yes | 1,732 (2.1) | 1,779 (1.3) | | |
| No | 81,272 (97.9) | 136,356 (98.7) | | |
| ***Substance use disorders*** | | | <0.0001* | 0.2756* |
| Yes | 16,340 (19.7) | 17,140 (12,4) | | |
| No | 66,664 (80.3) | 120,995 (87.6) | | |
| ***Autism spectrum disorder*** | | | <0.0001* | 0.2093* |
| Yes | 51 (0.1) | 56 (0.1) | | |
| No | 82,953 (99.9) | 138,079 (99.9) | | |
| ***Conduct disorder*** | | | <0.0001* | 0.2765* |
| Yes | 1,389 (1.67) | 1,348 (0.98) | | |
| No | 81,615 (98.3) | 136,787 (99.2) | | |
| **Maternal Health Care Use** | | | | |
| ***Maternal hospitalization*** | | | <0.0001* | 0.2509* |
| At least once | 20,849 (25.1) | 21,208 (15.4) | | |
| no | 67,155 (74.9) | 116,927 (84.6) | | |
| ***Number of maternal visits*** | 10.8 ± 6.6 | 8.6 ± 6.0 | < 0.0001* | 0.3539* |
| **Child Health Care Use** | | | | |
| ***Child hospitalization*** | | | <0.0001* | 0.0967 |
| At least once | 34,079 (41.1) | 47,391(34.3) | | |
| No | 48,925 (58.9) | 90,744 (65.7) | | |
| ***Number of child visits*** | 37.7 ± 30.3 | 31.1 ± 25.6 | < 0.0001* | 0.2389* |
| **Year of birth** | | | <0.0001* | 0.0045 |
| *1996* | 5,408 (6.5) | 8,304 (6.0) | | |
| *1997* | 5,067 (6.1) | 7,953 (5.8) | | |
| *1998* | 4,829 (5.8 | 8,043 (5.8 | | |
| *1999* | 4,912 (5.9) | 7,737 (5.6) | | |
| *2000* | 4,759 (5.7) | 7,693 (5.6) | | |
| *2001* | 4,888 (5.9 | 7,526 (5.5) | | |
| *2002* | 7,682 (5.6) | 4,620 (5.6) | | |
| *2003* | 4,413 (5.3) | 7,797 (3.5) | | |
| *2004* | 4,282 (5.2) | 7,723 (5.6) | | |
| *2005* | 4,506 (5.4) | 7,881 (5.7) | | |
| *2006* | 4,669 (5.6) | 8,147 (3.7) | | |
| *2007* | 5,042 (6.1) | 8,453 (6.1) | | |
| *2008* | 5,127 (6.2) | 8,603 (6.2) | | |
| *2009* | 5,186 (6.3) | 8,890 (6.4) | | |
| *2010* | 5,127 (6.2) | 8,796 (6.4) | | |
| *2011* | 5,305 (6.4) | 8,570 (6.2) | | |
| *2012* | 4,864 (5.9) | 8,337 (6.0) | | |

*Statistically significant

**Appendix 7- Bivariate analysis of follow-up time in the postnatal cohort**

| **Population** | *Mean± SD (years)* **Exposed** (N=171,230) | *Mean± SD (years)* **Non-exposed** (N= 49,909) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| Whole population | 10.2 ± 5.1 | 8.3 ± 4.9 | <0.0001* | 0.3842* |
| Diagnosed with mood and anxiety disorders | 15.1 ± 3.5 | 15.0 ± 3.5 | 0.5546 | 0.0286 |
| Censored due to death | 7.7 ± 5.5 | 6.8 ± 5.0 | <0.0001* | 0.1671* |
| Censored due to migration | 4.2 ± 3.7 | 3.5 ± 3.3 | <0.0001* | 0.1927* |

*Statistically significant

**Appendix 8- Distribution of additional covariates between exposed and non-exposed children during the first 3 years of life (N= 221,139 mother-infant dyads)**

| **Characteristics** | n (%)<br>Mean ± SD<br>**Exposed**<br>(N= 171,230) | n (%)<br>Mean ± SD<br>**Non-exposed**<br>(N= 49,909) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| **Child Medical Comorbidities** | | | | |
| ***Inflammatory bowel disease*** | | | <0.0001* | 0.0186 |
| Yes | 108 (0.1) | 10 (0.1) | | |
| No | 171,122 (99.9) | 49,899 (99.9) | | |
| ***Asthma*** | | | <0.0001* | 0.3548* |
| Yes | 39,852 (23.3) | 4,597 (9.2) | | |
| No | 131,378 (76.7) | 45,312 (90.8) | | |
| ***Cancer*** | | | <0.0001* | 0.0323 |
| Yes | 999 (0.6) | 174 (0.4) | | |
| No | 170,231 (99.4) | 49,735 (99.6) | | |
| ***Atopic dermatitis*** | | | <0.0001* | 0.3114* |
| Yes | 48,072 (28.1) | 7,112 (14.3) | | |
| No | 123,158 (71,9) | 42,797 (85.7) | | |
| ***Diabetes*** | | | 0.9181 | 0.0059 |
| Yes | 421 (0.3) | 121 (0.2) | | |
| No | 170,809 (99.7) | 49,788 (99.8) | | |
| ***Seizure disorders*** | | | <0.0001* | 0.3123* |
| Yes | 48,982 (28.6) | 7,269 (14.6) | | |
| No | 122,248 (71.4) | 42,640 (85.4) | | |
| ***Hypothyroidism*** | | | <0.0001* | 0.2143 |
| Yes | 734 (0.4) | 116 (0.2) | | |
| No | 170,496 (99.6) | 49,793 (99.8) | | |
| **Maternal History of Mental Health Disorders** | | | | |
| ***Attention deficit hyperactivity disorder*** | | | < 0.0001* | 0.1338* |
| Yes | 3,037 (1.8) | 625 (1.3) | | |
| No | 168,193 (98.2) | 49,284 (98.7) | | |
| ***Developmental disorders*** | | | 0.6362 | 0.0465 |
| Yes | 127 (0.1) | 33 (0.1) | | |
| No | 171,103 (99.9) | 49,876 (99.9) | | |
| ***Suicide attempt*** | | | < 0.0001* | 0.0875 |
| Yes | 4,317 (2.5) | 1,527 (3.1) | | |
| No | 166,913 (97.7) | 48,382 (96.9) | | |
| ***Mood and anxiety disorders*** | | | < 0.0001* | 0.2715* |
| Yes | 108,387 (63.3) | 23,867 (47.8) | | |
| No | 62,843 (36.7) | 26,042 (52.2) | | |
| ***Schizophrenia*** | | | < 0.0001* | 0.1327* |
| Yes | 1,269 (0.7) | 261 (0.5) | | |

| | | | | |
|---|---|---|---|---|
| No | 169,961(99.3) | 49,648 (99.5) | | |
| ***Psychotic disorders*** | | | 0.0009* | 0.0564 |
| Yes | 2,800 (1.6) | 711(1.4) | | |
| No | 168,430 (98.4) | 49,198 (98.6) | | |
| ***Substance use disorders*** | | | < 0.0001* | 0.1285* |
| Yes | 27,448 (16.0) | 6,032 (12.1) | | |
| No | 143,782 (84.0) | 43,877 (87.9) | | |
| ***Autism spectrum disorder*** | | | 0.9079 | 0.0257 |
| Yes | 84 (0.1) | 23 (0.1) | | |
| No | 171,146 (99.9) | 49,886 (99.9) | | |
| ***Conduct disorder*** | | | <0.0001* | 0.0971 |
| Yes | 2229 (1.3) | 508 (1.0) | | |
| No | 169,001 (98.7) | 49,401 (99.0) | | |
| **Maternal Health Care Use** | | | | |
| ***Maternal hospitalization*** | | | < 0.0001* | 0.0264 |
| At least once | 33,329 (19.5) | 8,728 (17.5) | | |
| no | 137,901 (80.5) | 41,181 (82.5) | | |
| ***Number of maternal visits*** | 9.6 ± 6.4 | 9.0 ± 6.1 | < 0.0001* | 0.0947 |
| **Child Health Care Use** | | | | |
| ***Child hospitalization*** | | | <0.0001* | 0.1652* |
| At least once | 67,089 (39.2) | 14,381 (28.8) | | |
| No | 104,141 (60.8) | 35,528 (71.2) | | |
| ***Number of child visits*** | 38.3 ± 28.6 | 17.5 ±16.0 | < 0.0001* | 0.7918* |
| **Year of birth** | | | <0.0001* | 0.1949* |
| *1996* | 11,852 (6.9) | 1,860 (3.7) | | |
| *1997* | 11,171 (6.5) | 1,849 (3.7) | | |
| *1998* | 10,912 (6.4) | 1,960 (3.9) | | |
| *1999* | 10,666 (6.2) | 1,983 (3.9) | | |
| *2000* | 10,415 (6.1) | 2,037 (4.1) | | |
| *2001* | 10,185 (6.0) | 2,229 (4.5) | | |
| *2002* | 9,974 (5.8) | 2,328 (4.7) | | |
| *2003* | 9,784 (5.7) | 2,426 (4.9) | | |
| *2004* | 9,553 (5.6) | 2,452 (4.9) | | |
| *2005* | 9,583 (5.6) | 2,804 (5.6) | | |
| *2006* | 9,634 (5.6) | 3,182 (6.4) | | |
| *2007* | 9,998 (5.8) | 3,497 (7.0) | | |
| *2008* | 10,303 (6.0) | 3,427 (6.9) | | |
| *2009* | 10,254 (6.0) | 3,822 (7.7) | | |
| *2010* | 10,026 (5.9) | 3,897 (7.8) | | |
| *2011* | 9,541 (5.6) | 4,334 (8.7) | | |
| *2012* | 7,379 (4.3) | 5,822 (11.7) | | |

*Statistically significant

**Appendix 9- Distribution of additional covaries amongst children with and without mood and anxiety disorders (N= 221,139 dyads)**

| **Characteristics** | n (%)<br>Mean ± SD<br>**Diagnosed**<br>(N= 8,854) | n (%)<br>Mean ± SD<br>**Not diagnosed**<br>(N=212,285) | ***p*-value** | **Std. Diff.** |
|---|---|---|---|---|
| **Child Medical Comorbidities** | | | | |
| ***Inflammatory bowel disease*** | | | <0.0001* | 0.4877* |
| Yes | 16 (0.2) | 102 (0.1) | | |
| No | 8,838 (99.8) | 212,183 (99.1) | | |
| ***Asthma*** | | | <0.0001* | 0.2942* |
| Yes | 3,814 (43.1) | 40,635 (19.1) | | |
| No | 5,040 (56.9) | 171,650 (80.9) | | |
| ***Cancer*** | | | <0.0001* | 0.3981* |
| Yes | 138 (1.6) | 1,035 (0.5) | | |
| No | 8,716 (98.4) | 211,250 (99.5) | | |
| ***Atopic dermatitis*** | | | <0.0001* | 0.3138* |
| Yes | 4,070 (46.0) | 161,171 (75.9) | | |
| No | 4,784 (54.0) | 51,114 (24.1) | | |
| ***Diabetes*** | | | <0.0001* | 0.6447* |
| Yes | 90 (1.0) | 452 (0.2) | | |
| No | 8,764 (98.9) | 211,833(99.8) | | |
| ***Seizure disorders*** | | | <0.0001* | 0.2575* |
| Yes | 4,356 (49.2) | 51,895 (24.5) | | |
| No | 4,498 (50.8) | 160,390 (75.5) | | |
| ***Hypothyroidism*** | | | <0.0001* | 0.5906* |
| Yes | 132 (1.5) | 718 (0.3) | | |
| No | 8,722 (98.5) | 211,567 (99.7) | | |
| **Maternal History of Mental Health Disorders** | | | | |
| ***Attention deficit hyperactivity disorder*** | | | <0.0001* | 0.0954 |
| Yes | 214 (2.4) | 3,448 (1.6) | | |
| No | 8,640 (97.6) | 208,837 (93.4) | | |
| ***Developmental disorders*** | | | 0.0391* | 0.1785* |
| Yes | 12 (0.1) | 148 (0.1) | | |
| No | 8,842 (99.9) | 212,137 (99.9) | | |
| ***Suicide attempt*** | | | <0.0001* | 0.1211* |
| Yes | 369 (4.2) | 5,475 (2.6) | | |
| No | 8,485 (95.8) | 206,810 (97.4) | | |
| ***Mood and anxiety disorders*** | | | <0.0001* | 0.1696* |
| Yes | 7,057 (79.7) | 125,197 (59.0) | | |
| No | 1,797 (20.3) | 87,088 (41.0) | | |
| ***Schizophrenia*** | | | <0.0001* | 0.2711* |

| | | | | |
|---|---|---|---|---|
| Yes | 142 (1.6) | 1388 (0.7) | | |
| No | 8,712 (98.4) | 210,897 (99.3) | | |
| ***Psychotic disorders*** | | | <0.0001* | 0.2015* |
| Yes | 277 (3.1) | 3,234 (1.5) | | |
| No | 8,577 (96.9) | 209,051 (98.5) | | |
| ***Substance use disorders*** | | | <0.0001* | 0.1627* |
| Yes | 2,245 (25.4) | 31,235 (14.7) | | |
| No | 6,609 (74.6) | 181,050 (85.3) | | |
| ***Autism spectrum disorder*** | | | 0.3271 | 0.0818 |
| Yes | 6 (0.1) | 101 (0.1) | | |
| No | 8,848 (99.9) | 212,184 (99.9) | | |
| ***Conduct disorder*** | | | 0.3511 | 0.0178 |
| Yes | 119 (1.3) | 2618 (1.2) | | |
| No | 8,735 (98.7) | 209,667 (98.8) | | |
| **Maternal Health Care Use** | | | | |
| ***Maternal hospitalization*** | | | <0.0001* | 0.2108* |
| At least once | 2,492 (28.2) | 39,565 (18.6) | | |
| no | 6,362 (71.8) | 172,720 (81.4) | | |
| ***Number of maternal visits*** | 8.4 (6.1) | 9.5 (6.3) | <0.0001* | 0.1754* |
| **Child Health Care Use** | | | | |
| ***Child Hospitalization*** | | | <0.0001* | 0.6301* |
| At least once | 5,220 (59.0) | 76,250 (35.9) | | |
| No | 3,634 (41.0) | 136,035 (64.1) | | |
| ***Number of child visits*** | 69.9 (41.5) | 32.1 (25.8) | <0.0001* | 1.4193* |
| **Year of birth** | | | <0.0001* | 0.6691* |
| *1996* | 2,015 (22.8) | 11,697 (5.5) | | |
| *1997* | 1,614 (18.2) | 11,406 (5.4) | | |
| *1998* | 1,304 (14.7) | 11,568 (5.5) | | |
| *1999* | 948 (10.7) | 11,701 (5.5) | | |
| *2000* | 786 (8.9) | 11,666 (5.5) | | |
| *2001* | 555 (6.3) | 11,859 (5.6) | | |
| *2002* | 465 (5.3) | 11,837 (5.6) | | |
| *2003* | 391(4.4) | 11,819 (5.6) | | |
| *2004* | 262 (3.0) | 11,743 (5.5) | | |
| *2005* | 207 (2.3) | 12,180 (5.7) | | |
| *2006* | 113 (1.3) | 12,703 (6.0) | | |
| *2007* | 74 (0.8) | 13,421(6.3) | | |
| *2008* | 80 (0.9) | 13,650 (6.4) | | |
| *2009* | < 10 [$] | 14,047 (6.6) | | |
| *2010* | 10 (0/1) | 13,913 (6.6) | | |
| *2011* | < 10 [$] | 13,874 (6.5) | | |
| *2012* | < 10 [$] | 13,201 (6.2) | | |

*Statistically significant

[$] Exact total number is suppressed due to privacy

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